Reprinted from the BOTANICAL GAZETTE, Vol. XXXVI, July and August 1903.

CONTRIBUTIONS FROM THE BOTANICAL LABORATORY OF THE JOHNS HOPKINS UNIVERSITY, No. 1.

ON THE GAMETOPHYTES AND EMBRYO OF TAXODIUM

A DISSERTATION
SUBMITTED TO THE BOARD OF UNIVERSITY STUDIES OF
THE JOHNS HOPKINS UNIVERSITY,
JUNE 1901
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

W. C. COKER

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VOLUME XXXVI NUMBER 1

BOTANICAL GAZETTE

JULY, 1903

ON THE GAMETOPHYTES AND EMBRYO OF TAXODIUM.[1]

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W. C. COKER.

(WITH PLATES I–XI)

IN spite of the recent great increase in our knowledge of spermatogenesis in many groups of gymnosperms, this part of the life history of the Taxodieae remained, at the time this work was undertaken, almost unknown. A short contribution by Shaw ('97) on Sequoia had appeared in 1897, and Arnoldi ('99[a, b]) has recently added two papers on the development of the reproductive organs in Sequoia. These observers have cleared up many salient points in the development of this genus, but the group as a whole is still to be studied.

Taxodium itself, probably on account of its limited geographical distribution, has been greatly neglected by investigators. Coulter on the histology of the leaf, Masters on the seedling, Lotsy and Meehan on the knees, and Von Schrenk on the disease called "peckiness" are among the few papers that have been devoted, in whole or in part, to the study of Taxodium, and none is concerned with the development of the seed.

The present work was suggested by Dr. D. S. Johnson, to whom I wish to express my gratitude for his unfailing kindness and helpful advice throughout its prosecution. I also wish

[1] A dissertation submitted to the Board of University Studies of the Johns Hopkins University, June 1901, for the degree of Doctor of Philosophy.

publicly to thank my brothers for their assistance in sending me material at frequent intervals.

METHODS.

Collections of *Taxodium distichum* Richard, the only species studied, have been made for about three years, chiefly from Hartsville, S. C., but also from Baltimore, Md., and New Berne, N. C. Fixing has been done at the tree in all critical stages, but fresh material, sent in tight boxes from Hartsville to Baltimore, has frequently given good results. Flemming's strong solution, chrom-acetic acid solution, alcoholic solution of picric acid, saturated solution of corrosive sublimate in 95 per cent. alcohol have all been used to some extent; but a saturated aqueous solution of corrosive sublimate (95 or 90 parts) and glacial acetic acid (5 or 10 parts) has been generally used. The latter gives results that are scarcely, if at all, inferior to those obtained with the Flemming solution, while it is more satisfactory than any of the other fluids mentioned. In searching for protoplasmic connections between cells, Gardner's ('83) methods were used, but only with fixed material. Potassium iodid and chlor-zinc-iodid were useful in determining the presence of starch, and have been used throughout for this purpose. A number of stains have been tried, but Flemming's triple has been most used. Young cones were split, or the scales removed entire. In older cones the ovule was removed and the nucellus exposed by breaking off the lignified tip of the integument, or the whole prothallium was taken from the seed. Sections 5–10 μ thick were made by the usual paraffin method.

THE STAMINATE CONE.

The staminate flowers are born on short branches which are either simple or compound. If simple, these branches are usually longer and more numerous than if compound. They appear in the fall from near the tips of the branches of the same year, and at the beginning of October or even earlier the young staminate flowers may be seen in the axils of their scale-like leaves. A longitudinal section of a sporophyll at this time shows no distinction between primary archesporium and other

tissue, all the cells of the lower part of the sporophyll being of about the same size, and having dense contents. Soon, however, certain centrally placed hypodermal cells begin to divide by periclinal walls and give rise to rows of cells as shown in *fig. 1*. The outermost cells of these rows, by a periclinal division, form the one-layered tapetum and the inner layer of the sporangial wall. By division of adjoining cells the tapetal layer is extended completely around the sporogenous tissue (*fig. 2*), and by January, or earlier, the microspore mother-cells are formed and ready for their division in early spring. Chamberlain ('98) has reported a similar stage during winter in the microsporangia of Pinus, Cupressus, and Taxus. The cells of the whole sporophyll, with the exception of the tapetum and the sporogenous tissue, contain starch through the late fall and winter until renewed growth in spring alters its arrangement. In the middle of November the nuclei of the tapetal layer show a peculiar structure not found at other times. They have a very coarse and wide-meshed reticulum, upon which the chromatin is distributed in large granules of very unequal size. There is no nucleolus. The nuclei of the sporogenous tissue have several nucleoli and a thinner reticulum than at a later stage.

No trace of an indusium-like outgrowth from the sporophyll is present for the protection of the sporangia, such as occurs in Cupressus, Thuja, and species of Juniperus. During early stages of development the cells of the upper part of the sporophyll are completely filled with a peculiar homogeneous substance staining bluish with gentian, which, as its subsequent history shows, is either a form of starch or an intermediate product in the formation of starch. It is not stained blue by iodin. At the stage of *fig. 1*, this substance is being replaced by starch grains of the usual kind, and a direct relation in amount between the two is evident, the starch appearing in proportion as the amorphous substance disappears. The cells on the line of transformation contain both starch and amorphous substances in proportionately smaller quantities.

Before their division in the spring, the pollen mother-cells become filled with starch, while the grains in other parts of the

sporophyll are being rapidly corroded. The persistence of this starch in the mother-cells during division and its disappearance as the exine is formed in the pollen grain agrees with what is already known in cycads and conifers. The ripe pollen grain contains no starch, nor is any found in the pollen tube until it appears in the protoplasm of the central cell shortly before the formation of the sperm cells. The number of microsporangia on a sporophyll may be as many as nine, seven being a common number. The wall of the mature microsporangium consists of but two layers of cells on the exposed surface, and in this respect Taxodium differs from the Abieteae, Taxeae, Cycadales, and Ginkgo, and agrees with the Cupresseae and Gnetales. The cells of the outer layer of the wall have the sides and inner faces strengthened by bands of cellulose, while those of the inner layer are very much flattened and poor in contents. The cells in the tapetum have very dense contents and are shorter and thicker than those of the inner wall. They disorganize at about the time that the division occurs in the pollen grains.

The division of the pollen mother-cells took place this year (1901) in South Carolina on March 6th, and both divisions were found on the same day, even in the same cone, but the stages found in the same sporangium are not quite so different as Coulter and Chamberlain ('01) figure for *Pinus Laricio*. Changes of the nucleus leading up to the first division were not present in my material, but good preparations of all stages during and subsequent to the metaphase of the first division show that the phenomena are similar in all essential respects to those described in detail by Strasburger ('00) for Larix.

The chromosomes, as arranged on the nuclear plate, are short and thick (*fig. 3*). They stand at right angles to the axis of the spindle, the fibers being attached to the inner ends. The splitting begins at the point of attachment and in favorable cases the line may be seen between the two halves in the as yet unseparated outer limb. Very soon after the splitting is completed and the daughter chromosomes begin to move to the poles, the fibers are seen to be attached to the middle of the

bent chromosomes and the inner ends of the latter are composed of four arms, lying side by side, and generally of the same length (*figs. 4–6*). The second splitting has evidently occurred and the arrangement is now just as in Larix as described by Strasburger ('00). The chromosomes remain thick and short as they approach the poles, and their number can be easily determined to be either eleven or twelve. Eleven are shown in *fig. 6* in polar view, and at least this number could be distinctly made out in other cases. Sometimes there seem to be twelve, but on account of the crowding in such cases I have never been sure of this number. Twelve chromosomes have been found by Belajeff ('94) and Strasburger ('92) in the pollen mother-cells of *Larix europaea*, by Blackmann ('98) in pollen mother-cells and oosphere of *Pinus sylvestris*, by Juel ('00) in the megaspore mother-cell of *Larix sibirica*, and by Chamberlain ('99) in the pollen mother-cells, endosperm, and jacket cells of *Pinis laricio*. It would thus seem from analogy that the number of chromosomes in the pollen mother-cells of Taxodium is also twelve rather than eleven.

The daughter nuclei (*fig. 7*) before the next division enter into a fairly well-developed resting stage. There is a distinct reticulum, if indeed a rather coarse one, and the chromatin is grouped in larger masses than in the reticulum of many resting cells, approaching more nearly the condition already described in the nuclei of the tapetal layer of the microsporangium in November. Strasburger ('00) describes such a condition in Larix, but tries to bring it in harmony with other cases by considering the network as spun out from the chromosomes. His distinction is not clear to me, and I think it must be acknowledged that the daughter nuclei of the first division may, at least in some cases, reach before their next division a relatively well advanced resting stage. From *fig. 7* it will be seen that the cell walls of the mother-cell have not disappeared at the time of tetrad-formation. In places the walls have begun to go to pieces, but in others remain entire and in close contact with their neighbors. No case was found where the final divisions were bilateral, as is sometimes the case in *Pinus Laricio* (Coulter and Chamberlain, '01).

The connecting fibers of the first spindle produce a distinct cell-plate, extending entirely across the cell before the nuclei have begun to divide a second time. On each side of the plate the starch grains are densely crowded. The chromosomes of the second division are single slightly curved rods, and are evidently of about the same size as the halves of the double chromosomes of the first division. The starch begins to disappear during the second division of the pollen mother-cell, and is completely used up during the formation of the exine of the pollen grains, which becomes quite evident in about three days after the last division. The nucleus of the fully formed but yet undivided pollen grain is evenly and coarsely granular and generally without a nucleolus (*fig. 8*).

About ten days after its formation the pollen grain divides. The spindle is very small and the chromosomes are proportionately longer than in the reducing division (*figs. 9* and *10*). This is the only division of the pollen grain, no sterile prothallial cell being formed, and it separates at once the generative cell from the tube cell. The former is flattened lens-shaped, concave toward the inside, and furnished with a distinct *Hautschicht* (*fig. 11*). This division occurs a few days before the pollen is shed, and it is in this condition that the ripe pollen reaches the nucellus (*fig. 12*). In the absence of any sterile prothallial cells, Taxodium agrees with the Cupresseae and Taxus, and differs from all other conifers and cycads. The number of sterile prothallial cells in the pollen grain of gymnosperms has been determined in the following cases: two in Ginkgo (Strasburger, '92), *Larix europaea* (Strasburger, '84), *Picea vulgaris* (Belajeff, '93), *Pinus silvestris* (Strasburger, '92), *Pinus Pumilio* (Coulter and Chamberlain, '01); one in Ceratozamia (Juranyi, '82; he occasionally found two in *C. longifolia*), Zamia (Webber, '97), Cycas (Ikeno, '99); none in Biota, Cupressus, Juniperus (Strasburger, '92), *Taxus baccata* and Juniperus (Belajeff, '93).

The great importance of correctly determining the number of divisions in the pollen grain has not been overlooked, and repeated sections, at all stages of the development of the pollen

from the mother-cell stage to the sprouting of the pollen tube, have been made from collections obtained in both 1900 and 1901, and I think it certain that there is but one division of the pollen cell in Taxodium.

THE POLLEN TUBE.

The first indication of sprouting is given by the swelling up of the generative cell into the tube cell, and by an increase in size of both nuclei (*fig. 13*). The exine is usually thrown off at an early stage, as shown in *fig. 14*. In this figure the nuclei of the pollen tube have not changed their position, the tube nucleus lying immediately above the generative cell. The pollen tube contains no starch, either now or during its course to the prothallium. As the tube advances, the tube nucleus moves from its position over the generative cell and passes slowly down toward the tip. Indications of branching are soon seen in the pollen tube (*figs. 17, 29, 22, 23*). In *fig. 16* the generative cell seems by its position to be bounded by an actual membrane, but no indication of a cellulose wall was obtained, and if one is present it is exceedingly thin and quickly dissolved. By comparing *figs. 15* and *16* it will be seen that the sprouting does not take place at any definite point in reference to the position of the generative cell.

The division of the generative cell does not occur until several weeks after the sprouting of the grain (*figs. 19–21*). The stalk nucleus soon loses its definite hold upon the protoplasm around it, although immediately after the division (*fig. 19*) it is still bounded by a distinct protoplasmic sheath. The central cell retains the characteristics which mark the generative cell before division. It is furnished with a distinct *Hautschicht* and has the shape of a double convex lens. It will be noticed that immediately after the division the stalk cell is larger than the central cell. Belajeff ('91, '93) describes these two cells as being of equal size in Taxus. In *Juniperus communis* he ('93) finds the outer cell to be smaller, while in *Picea vulgaris* the opposite is true. There is not much difference in size in *Pinus Laricio*, as figured by Coulter and Chamberlain ('91). It will thus be seen that Taxodium agrees with Juniperus in the relative size of the stalk and central cells immediately after their formation.

Belajeff ('91, '93) describes the stalk nucleus as passing the central cell as it wanders down the tube. Such a description could hardly be applied in Taxodium when the tube is at right angles to the axis of the spindle of the generative cell. The stalk nucleus is as near the tip of the tube as is the central cell, and they both wander down together until they reach the tube nucleus (*fig. 22*). It will be seen from *fig. 26* that the three nuclei of the pollen tube can easily be distinguished at this stage. The stalk nucleus is smaller than the tube nucleus, while the protoplasm of the central cell is distinct from that of the pollen tube. The stalk and tube nuclei now advance slightly ahead of the central cell (*fig. 23*), and this relative position is retained by the three nuclei throughout the subsequent history of the pollen tube. In *fig. 23* the stalk nucleus is still slightly smaller than the tube nucleus, but the structure of the two is the same. The male nucleus is very like the other two, its nucleolus being slightly smaller.

The pollen tube proceeds to the prothallium without interruption; the growth, however, is much slower in the upper part of the nucellus than in the lower. No particular tissue of the nucellus tip is set apart to nourish or guide the pollen tube. All of its cells contain more or less starch, but there is no grouping of starch in definite areas. The pollen tube may reach the megaspore before the formation of a cellular prothallium (*fig. 25*). So early an approach of the pollen tube to the sprouting megaspore has not been described in any other case, so far as I am aware. Jäger ('99) gives one figure of *Taxus baccata* showing a pollen tube almost in contact with a young prothallium, and I have found that in *Taxus baccata canadensis* the pollen tube may reach the level of the megaspore before the latter has divided even once. One case was found in this plant where the pollen tube has grown against and badly compressed the megaspore before the latter had advanced far beyond the sixteen-cell stage. It was so completely crushed that the stage could not be exactly determined.

Fig. 26 gives the structure of the contents of the pollen-tube at a slightly later stage than *fig. 25*. The two free nuclei are

now exactly similar and lie side by side immediately beneath the central cell. The latter has increased greatly in size, as has also its nucleus, and the protoplasm is seen to possess a radiate structure. We find in the nucleus of the central cell a distinct peripheral network, and a nucleolus, irregular in outline and evidently of a compound nature. This kind of nucleolus, which we here meet for the first time, will be found to occur also in the nucleus of the central cell of the archegonium. In one case the central cell was at the tip of the pollen tube, with the two free nuclei behind it. One of the latter was pressed so closely to the protoplasm of the central cell as to indent it slightly. Such an abnormal relation between the generative and free nuclei has been noted in *Pinus Laricio* by Coulter ('97).

The further changes in the central cell before its final division into the sperm cells are so remarkable and have been so neglected in other conifers studied that I shall go into them with some detail. *Fig. 27* represents the central cell after it has reached its full size. It is no longer spherical, but has become elliptical in section, the long axis being perpendicular to the axis of the tube. The protoplasm is seen to be radiating from the two poles of the long axis. At these poles are sometimes to be distinguished slightly more granular areas, from which the radiations seem to diverge. The protoplasm is very dense, finely granular and in thin sections can be seen to have a reticulate structure. The faint areas at the poles of the cells will at once suggest in position the blepharoplasts of Ginkgo, Zamia, and Cycas. In reality, the resemblance is entirely confined to their position. Dr. Webber has kindly shown me his preparations of blepharoplasts in Zamia, and their intense staining and large size make further comparison impossible.

The nucleus, which is about half of the diameter of the cell, has rather abundant reticulum and a fragmented nucleolus. In addition to these, there has appeared a finely granular material which does not seem to differ in any respect from the linin material in the egg nucleus to be described below. It is most abundant around the nucleolus, but extends to all parts of the nucleus. In *fig. 27* one of the free nuclei is seen closely appressed

to the *Hautschicht* of the central cell; the other free nucleus does not appear in the section. This is about the latest stage at which these free nuclei retain their normal structure. They very soon begin to go to pieces, and the protoplasm of the pollen tube at the same time begins to disorganize. It becomes more homogeneous and retains more tenaciously the safranin stain. The nucleoli and chromatin of the free nuclei become more or less broken up and collected into masses of different size, a process which we shall see corresponds exactly to what occurs in the nuclei of the jacket cells of the archegonium shortly before its final division. Concomitantly with the disorganization of the nuclei and cytoplasm of the pollen tube, there becomes evident in the cytoplasm of the central cell a number of bodies staining a deep red in safranin. They resemble exactly the plastin granules that we have seen to appear at the disorganization of the free nuclei, and that they are actually transferred from the latter into the central cell seems possible. *Fig. 28* is a central cell after the appearance of these granules. They are arranged in a circular manner at some distance from the nucleus, and it may be that this distribution is connected in some way with the concentric arrangement of the fibers. At the time of the appearance of the plastin granules in the protoplasm of the central cell, there seems to be a distinct connection at the base of the cell between its protoplasm and that of the disorganizing material beneath it. Hirase ('95) describes large bodies lying in the protoplasm of the central cell of Ginkgo between the nucleus and the blepharoplasts. Webber ('97) confirms this and says that in addition to the two large bodies smaller masses of similar material were observed in other localities of the cell. He speaks of them as extra-nuclear nuclein. It is easy to compare these bodies with those of Taxodium. They stain deeply with safranin in both cases, the principal difference being that in Ginkgo they are generally fused into two large masses which occupy a definite position in the cell.

The disorganized mass of nuclei and protoplasm at the tip of the tube never completely disappears before fertilization, and it may appear in the tip of the archegonium above the protoplasm

of the egg after fertilization. In *fig. 28* a number of scattered starch grains have made their appearance in the cytoplasm of the central cell. They retain their scattered position until finally arranged into the dense starch sheath immediately surrounding the nucleus of the sperm-cell.

Changes in the nucleus preparatory to the final division of the central cell had already begun at the stage represented in *fig. 27*. In *fig. 28* these changes have proceeded still further. The chromosomes are being prepared from the few thick conspicuous threads that are present. The linin granules have become organized into a reticulum, and this reticulum seems to be arranging itself as if in the preparatory stages of spindle formation.

No attempt was made to study in detail all stages of spindle formation in the division of the central cell, but in *fig. 29*, which shows an oblique view of the spindle, the formation of its fibers from the nuclear reticulum and the granular nature of the more peripheral fibers seems evident. This formation of the spindle from the fibers of the nucleus will be described in more detail in the division of the ventral canal cell. *Fig. 30* shows a late telophase in the division of the central cell, the connecting fibers still being evident. A clear area is noticed on each side of the cell plate, and this area later extends entirely around the sperm cells. The starch and plastin material are collected at the distal ends of the spindle, but after the separation of the two daughter cells the starch becomes arranged in a dense sheath immediately surrounding the nucleus (*fig. 31*). Just outside of this sheath the plastin granules form a more or less complete layer. Beyond them is found the clear area previously mentioned, and the surface is composed of a distinct membrane which sharply defines the sperm cells from the protoplasm of the tube. After the formation of the daughter nuclei, they again begin to fill with the linin granules or reticulum (the so-called metaplasmic substance of Strasburger) until, at the time of maturity, they are so dense as to make any distinction between the granular material and chromatin reticulum very difficult. A small nucleolus, however, can be dimly discerned (*fig. 31*).

The sperm cells are now mature, and fertilization almost immediately takes place. I think it probable that the sperm cells do not round themselves off completely until after the bursting of the pollen tube, for although sometimes separated as much as a quarter of their diameter from each other, I have never seen them while still in the tube without a flat face on the inner side. This remarkably complex structure of the sperm cell distinguishes Taxodium from any phanerogam hitherto described, with the exception of Ginkgo and the cycads.

Recent work on the conifers, which in the structure of the male gametophyte approach Taxodium, gives very little detail as to the structure of the sperm cells. In Taxus Jäger ('99) mentions radial striae in the periphery of both the sperm cell and its smaller, functionless sister cell, but gives no further details of the protoplasmic structure. Arnoldi ('98) says that in Cephalotaxus the protoplasm of the sperm cells, which are here of equal size, is densest around the nucleus. In his work on Sequoia ('99) he gives no details of the protoplasmic structure of the sperm cells, but says they resemble those of the Cupresseae. Blackman ('98) makes some interesting observations on the sperm cells of *Pinus silvestris*. He says: "It cannot be doubted that cytoplasm also passes over into the oosphere, for each generative nucleus in the pollen tube is clearly surrounded by its own layer of cytoplasm, as can be observed in the stage when the tube is already in contact with the oosphere." Also, "it may here be noticed that small bodies staining deeply with fuchsin S may be observed in the generative cell protoplasm." These, he says, resemble leucoplasts. "If leucoplasts are really present in the cytoplasm belonging to the generative cells, the general view that the male cell brings over no plastids to the egg appears to be directly contradicted." This is the only mention I have seen made of a distinctive protoplasm belonging to the male cells in any of the Abieteae.

Neither Belajeff ('91, '93) nor Strasburger ('79, '84) describe the structure of the sperm cells of the Cupresseae in detail, but in gross structure they seem almost identical with those of Taxodium. Strasburger ('84) says that the pollen tube of Juni-

perus contains very little starch at time of fertilization, and thinks it therefore the more remarkable that the fusion nucleus should be surrounded by so much starch. From comparison with Taxodium, however, it seems probable that starch is also present in the sperm cells of the Cupresseae and has heretofore been overlooked. We shall see that a comparison of the processes of fertilization in the two cases strengthens this view.

The tip of the pollen tube has not been found to possess a distinct pit, such as is described by Goroschankin ('83), Dixon ('94), and Blackman ('98) in other conifers. The whole tip of the tube is furnished with a thinner cell wall than is found above, and if any pit occurs it is rendered less conspicuous by the thinness of the adjoining wall.

THE OVULATE CONE.

In October of the year preceding the ripening of the seed, the ovulate cones of Taxodium appear as very inconspicuous axillary buds on shoots of the same year. They usually occupy positions near the tip of the branch, and vary greatly in the number formed. It has usually been said that the ovulate cones are borne two or three together at the tip of branches which have also produced, further down, branches of the staminate inflorescence. While this is sometimes the case, and frequently so in trees examined in Baltimore, in its more natural habitat the ovulate cones are situated on branches of their own, and occur in much greater numbers than described. As many as fifteen or twenty mature cones have been found closely crowded on a fertile branch, and while this is the exception, as many as eight or ten are frequently grouped on vigorous trees. The ovulate cones replace the dehiscent short branches. The latter do not appear in the axils of all the scale leaves during the first year, but in only about one-third of them. The next year they are found in axils of leaves which were not occupied the previous year, but in following years they come from supernumerary axillary buds, and in the more slowly growing parts of the tree may appear year after year in the axil of the same scale leaf.

Fig. 32 shows a megasporophyll collected October 3, 1899.

In the axil of the sporophyll two swellings are present, the section passing longitudinally through one of them. These are the rudiments of the ovules which by January 4 (*fig. 33*) have begun to show the first indication of an integument. *Fig. 34* shows a sporophyll collected in Baltimore March 11. The ovule has increased in size and the nucellus and integument are of equal height. It seems that slow growth is continued all through the winter months whenever the condition of the weather will permit. A few weeks before the time of pollination, the placental outgrowth begins to appear as slight projections between and at the sides of the sporangia. By April 8 (*fig. 36*) the cushion has begun to show between ovule and scale, and by April 22 (*fig. 37*) it has reached a considerable size. The further development of the sporophyll is almost entirely confined to its basal part where cushion and scale are indistinguishable, and to the great extension of the cushion above and sidewise as a protective covering to the ovules. The tip above the cushion remains small and is soon much surpassed by the latter, which by fusing with the scale above soon comes to inclose completely the cavity in which the ovules lie. The outgrowths are not confined to the ovule-bearing scales, but are developed in almost as great degree in the axils of the adjoining scales below and at the tip. The number of fertile scales is usually about ten. They are bounded beneath and above by scales differing from them only in the absence of ovules. As the growth proceeds, the area of attachment of the ovules to the scale becomes much greater in extent, so that when the seed is mature almost the whole of its outer face is attached to the inner face of the scale.

This is not the place for a discussion of the homologies of the so-called placental cushion, and I shall confine myself to the expression of my belief that it is a new formation for the purpose of closing the opening between the scales for the protection of the ovules, and is not derived either from fused leaves or from a second integument of the ovule.

At the time of pollination the tip of the integument is composed of about three layers of cells, but immediately after pol-

lination the inner cells near the tip begin to grow in at certain points, and approaching the center almost completely close the micropylar cavity (*fig. 37*). There soon begins to appear in the tip of the integument an irregular ring of lignified cells with thick and pitted walls, which serves to strengthen this exposed end (*fig. 49*).

THE MEGASPORE.

The megaspore mother-cell cannot be distinguished from its neighbors until shortly before pollination. It is the basal or next to the basal cell of one of the distinct cell-rows which are evident in the center of the nucellus, and is situated at the same level as the point of insertion of the integument (*figs. 35, 37*). In this respect Taxodium agrees with the Cupresseae and differs markedly from Sequoia (Shaw, '96). At the time of pollination the megaspore mother-cell is slightly larger than the cells immediately surrounding it. Only a single megaspore mother-cell is present at this stage, but one case was found (*fig. 50*) in which the nucellus contained two young prothallia, one of which was larger than the other. Whether these were derived from two mother-cells or from the same mother-cell is a matter of conjecture. In this respect also Taxodium is seen to differ from Sequoia, in which Shaw ('96) and Arnoldi ('99[b]) have found it the rule for a number of prothallia to be developed. Hofmeister ('51) mentions the occasional presence of two prothallia in *Pinus silvestris* and *Taxus baccata* (confirmed since by Farmer ['92] for Pinus and by Jäger for Taxus), and I have found two in Podocarpus and *Taxus baccata canadensis*. With these exceptions the development of more than one prothallium has not been observed in the conifers.

The mother-cell increases slowly in size after pollination, and in about ten days the first division occurs. *Fig. 39* is a mother-cell shortly before its first division. It is abundantly supplied with starch grains, as are also the adjoining cells for about one layer. It will be seen that the nucleus is in synapsis, the meshwork being in a contracted mass near the nucleolus. Such a synapsis is found by Juel ('00) at the same stage in *Larix sibirica*. He also mentions the presence of denser fibrous areas at

either end of the cell near the nucleus. These areas are frequently to be noticed in the megaspore of Taxodium, but I have not been able to establish any definite relation between them and the spindle-formation (*figs. 38–43*). A slightly younger stage is shown in *fig. 38*, where the nucleus is of the usual structure and has not approached the tip of the cell, in which position it is always to be found before the first division. Stages of the first division are shown in *figs. 40–42*. The chromosomes were not counted, but are evidently not far from twelve in number. This division cuts off a large lower cell and a much smaller upper cell. The lower cell immediately prepares to divide again. The second spindle is shown in *fig. 43*, and in *fig. 44* the division is complete. The starch has begun to disappear during these divisions, but some is present until the conclusion of the second. Strasburger ('79) describes it as disappearing in *Larix europaea* before the second division; the same is true in *L. sibirica* (Juel, '01) and *Pinus Pumilio* (Coulter and Chamberlain, '01).

The upper of the two cells formed at the first division does not divide again, and its nucleus never reaches the resting stage, or indeed approaches it. *Fig. 42* shows the difference in the nuclei of the upper and lower cells of the first division. The lower is developing as usual, but the upper has formed no reticulum, and in fact never reaches a more highly organized stage. Its chromosomes remain fused and lumped, and soon present merely a disorganized, homogeneous appearance. This history of the upper nucleus is repeated in detail by that of the upper cell of the second division. There are thus formed in Taxodium only three cells from the division of the megaspore mother-cell, but as the lower divides twice, it is in every respect the equivalent of a pollen grain, as much so as if the upper cell of the first division had divided, as is the case according to Juel ('00) in *Abies sibirica*. Strasburger ('79) gives three, or seldom more, as the number of potential megaspores derived from the mother-cell in Taxus. He also gives the same number in *Larix europaea*, but as Juel has found four in *L. sibirica* it is possible that Strasburger may have overlooked one in *L. europaea*. Coulter and

Chamberlain ('01) also report four potential megaspores in *Pinus Laricio*, and Shaw ('96) has given the number as four in Sequoia. Almost nothing is known of the divisions of the megaspore mother-cell in the Cupresseae beyond Strasburger's remark that in Thuja the origin of the prothallium is essentially as in Taxus.

THE LARGE-CELLED TISSUE OR TAPETUM.

The cells immediately adjoining the megaspore mother-cell, as before stated, contain starch (*fig. 39*). After the megaspore is formed and begins to increase in size, the number of these dense, starch-containing, much enlarged cells is found to be greater (*fig. 45*). In *fig. 39* the transition between the starch-containing cells and the ordinary tissue around them is not very abrupt, but in *fig. 45* the boundary between the two has become distinctly marked. The larger cells are in close contact, their cell walls are intact, and their nuclei are large and apparently perfectly normal. Indeed, one of them is dividing mitotically, and they are not infrequently found dividing at this stage. *Fig. 46* shows such a cell in division. The spindle is placed centrally in the cell — not at one end, as in the division of the spore mother-cell — and in one case a cell-plate is being formed. The chromosomes were not counted, but the number is much more than twelve. The division seems to be an ordinary typical one. The cells immediately beyond the large-celled tissues are distinctly flattened and seem to be crushed by the cells within. On the lower side the nuclei of the nucellar tissue are frequently small, deeply stained in safranin, and apparently going to pieces. In one case at about this stage the nuclei in this position had entirely disappeared for a distance of several layers beyond the normal large-celled tissue. *Fig. 47* shows the large-celled tissue at a later stage. It has increased greatly in amount and the cells, which are now slightly separated from each other, are much more numerous. They retain the characters mentioned above for a younger stage.

The nucellar cells bordering on this tissue now show unmistakable signs of disorganization. They are completely crushed and broken up and are as strongly distinct from the large-celled

tissue as the latter is from the megaspore. *Fig. 48* is a more magnified section of the same spore and tissue. A small amount of granular material appears between the spore and the large-celled tissue, but the inner walls of the latter are apparently intact. A few cases were found, however, where the innermost of the large cells seemed to be partly broken up on the side next the spore. At the stage shown in *fig. 49* the existence of so definite a layer around the spore is doubtful. The cells adjoining the spore are still larger than others beyond them, but in all cases that I have found they are more or less separated from each other and approach in appearance the loosened "spongy tissue" which has been described in other cases. In later stages, however, there constantly occurs a single layer of very large rectangular cells lying next to the megaspore, while beyond those the ordinary nucellus-tissue is crushed and disorganized. *Fig. 51* shows such a layer around a large spore before the formation of the cellular prothallium. The large cells seem perfectly intact; walls are present on both sides and the nucleus is large, has an abundant reticulum, and is more like what would be expected in an actively secreting cell than in a rapidly disorganizing one. *Figs. 52* and *53* show the same layer at a later stage, just before the prothallium has reached its full size. In *fig. 52* the wall of the megaspore has shrunken somewhat and the large cells have become more elongated and slightly separated. The layer of large cells and two or three layers of ordinary cells beyond it are always furnished with starch during the growth of the prothallium; the starch is continually accumulating in the further-removed cells as the inner ones are being disorganized. The large-celled layer is also destroyed at the last moment and the mature prothallium is surrounded at its upper end with four or five layers of ordinary nucellar cells. In the Abieteae the young germinating megaspore is imbedded in a loose tissue which resembles somewhat the large-celled tissue just described in Taxodium. Its limits are so distinct that Hofmeister ('62) mistook it for endosperm. In comparing Strasburger's ('79) figures of such tissue in Pinus and Larix with my *figs. 45* and *47* in Taxodium, it will be seen

that there is a great difference in structure in the two cases. Moreover, this tissue in the Abieteae is always spoken of as disorganizing. Coulter and Chamberlain ('01) have an interesting paragraph on this subject. They say: "In our figure of a mother-cell of *Pinus Laricio* imbedded in nucellar tissue, it is apparent that it is surrounded by a rather definite zone of cells, two to four layers in depth, which give evidence of breaking down. After endosperm-formation is somewhat advanced, this interesting zone becomes differentiated into two distinct regions, an outer layer of tabular, almost empty, cells, and an inner region of polygonal cells with densely staining contents." I do not know of any case, however, where this layer is said to persist in later stages. Strasburger ('79) describes a zone of more or less disorganized cells around the germinating megaspore of Thuja. I have examined growing sacs of Pinus, Larix, Thuja, Podocarpus, and Taxus in reference to this point. At the base of the prothallium in Podocarpus and around the very young germinating megaspore of Thuja there are cells which approach, in appearance, those found in Taxodium. In fact, there are frequently present around growing prothallia a number of swollen free cells, which might be compared to such a tissue as I have described in Taxodium, but in most cases these cells are not in close contact with one another and their development can be gradually traced from the ordinary cells of the nucellus. But I have not studied any of these plants carefully enough to deny the persistence in them of this tissue, and further observation is necessary to decide the matter.

It is difficult to understand the constant occurrence of a definite layer of large, distinctive, undisorganized cells around the growing prothallium in Taxodium, unless we ascribe to them an active part in the nourishment of the young gametophyte, and this I believe to be their real function. If this interpretation is the correct one, the tissue in question may be considered as a tapetum, which, instead of disorganizing at the maturity of the spore, as is usually the case, has continued its growth to keep pace with the developing prothallium which it continues to nourish until mature. If we consider the archesporium as

reduced to a single cell (the megaspore mother-cell), the tissue immediately around this cell must be considered as a tapetum, and we have seen that it is probably by the division of this tissue that the nourishing layer is formed. This cannot as yet be positively asserted for the later stages, as all steps have not been followed. The only other interpretation that seems possible is that the cells immediately surrounding the megaspore represent an originally archesporial tissue which has given up its function of spore-production and taken up the new rôle of nourishing the young plant within. Of these two interpretations I consider the first as much the more likely.

It is well known that the tapetum in the megasporangium of Selaginella persists intact until some time after the sprouting of the spores, which in this case are shed only after a considerable growth has occurred. Should the spores not be shed at all, and the tapetum continue still further its growth and function, we would have a condition paralleling that found in Taxodium.

DEVELOPMENT OF THE PROTHALLIUM.

We have left the megaspore to follow the surrounding tissue through its subsequent stages. The growth of the germinating megaspore is extremely slow during the first month after its formation, having reached on April 29 only about the 32-celled stage (*fig.* 47). Growth now becomes more rapid, and on May 6 it has reached the stage shown in *fig.* 49. About the beginning of June growth has stopped in the upper part and the formation of cell walls begins. *Fig.* 25 shows a stage just before the beginning of cell-formation. The pollen tube has already reached the megaspore, which now contains an enormous vacuole surrounded by a protoplasmic layer containing nuclei. In this case the protoplasmic layer has collapsed.

The great size reached by the germinating megaspore before the formation of cellular tissue seems to distinguish Taxodium from other conifers thus far studied. The wall of the spore was found to be furnished with pits. *Figs.* 97 and 98 show those pits in surface view at a time shortly before the formation of a cellular prothallium. The manner of the formation of the

first cell walls in the young gametophyte is shown in *fig. 24*, and I can confirm Mlle. Sokolowa's observation, also recently repeated by Ikeno ('98) and Arnoldi ('00), that the inner sides of the first cells are open. Mlle. Sokolowa gives the name "alveoli" to the ingrowing tubular cells of the prothallium, but this term does not seem to have much to recommend it. It is repeated by Arnoldi ('00) in his work on Sequoia.

In view of the supposed relationship of Taxodium and Sequoia, it is of interest to compare the endosperm-formation in the two cases. Arnoldi has described in some detail a double process in Sequoia which he considers to have significance from the phylogenetic point of view. The prothallial region which is to bear the archegonia is formed in the usual way by ingrowing open tubes, which finally meet in the center. This area may be either in the center of the sac alone, or may extend entirely to the tip. In the latter case, however, Arnoldi considers the tip to be lacking. The tissue at the tip does not form in the usual way, but it is produced by free cell-formation, as in the endosperm of angiosperms. This free cell-formation begins in the tips before the tubes appear in the archegonial region. In Taxodium, on the contrary, cell-formation usually begins earlier at the tip, where archegonia are to appear, than at the base of the prothallium, and firm cell walls are frequently lacking in the lower parts long after they have been established in the upper. In fact, cases are not infrequent in which the lower part of the gametophyte is in an embryonic condition, even after fertilization has occurred in the archegonia. In some cases, however, firm cell walls seem to be formed almost simultaneously throughout the spore. In the upper part of the prothallium it is always easy to see that cell-formation has proceeded by the usual growing-in method.

Fig. 55 shows prothallial tubes from near the tip of the sac after their closure on the inner side. (Mlle. Sokolowa ['90] has shown that the closure occurs when the inner ends of the tubes meet in the center.) *Fig. 56* shows the first division of a prothallial tube and the preparation for the second. By these divisions there are formed rows of cells radiating from the

center outward. *Fig. 57* represents the whole tip of a prothallium at the same stage as in *fig. 56*. Mlle. Sokolowa describes the nucleus of the open prothallial tube as remaining at the inner end during its growth toward the center, but after the formation of the closing walls the nucleus again moves back to near the periphery of the cell. From *fig. 54* we see that the first statement is true in Taxodium, at least during the very young stages. Good preparations were not obtained by me in older prothallial tubes before the closure. From Mlle. Sokolowa's figure of Juniperus, it seems that the mother-cells of the archegonia behave in this respect just as the other cells of the prothallium, and this is probably true in Taxodium also.

The prothallial formation in the lower part of the spore does not appear to me to be different in essentials from its formation above. It is only in the late development of cell walls, and not in their peculiar origin, that the difference consists. The lower part, even after the formation of cell walls, continues to increase in size long after the upper part has ceased to grow. In the ripe seed the upper end of the nucellus is no larger than at the time of fertilization, while the lower part increases greatly in size, the whole prothallium acquiring the shape of a slightly bent club. After a certain number of cell walls are formed in the prothallial tubes, nuclear divisions occur without the formation of cell walls, and there arises a multinucleate condition (*figs. 58, 59*). These nuclear divisions are generally, at least, of the mitotic type. Jäger ('99) describes a fusion of nuclei in the prothallial cells of Taxus, but I have not found the number of nuclei appreciably smaller in Taxodium even after the formation of the embryo.

DEVELOPMENT OF THE ARCHEGONIA.

The archegonia of Taxodium are disposed exactly as in the Cupresseae. They form a compact group at the base of a common depression in the center of the tip of the prothallium. Among the many hundred prothallia sectioned only three or four were found in which any variation in this arrangement occurred. In these exceptional cases there were several smaller

groups of archegonia which were separated by a few layers of prothallial cells. They were always situated at the tip of the prothallium, but sometimes faced to one side.

In *fig. 57* the initial cells of the archegonia are shown just before the cutting off of the neck cell. The nucleus is situated at the very tip of the cell and most of the protoplasm is collected around it. A very large vacuole occupies the greater part of the archegonium. In *fig. 60* the neck cell is being cut off. The nuclei in the central initials are preparing to divide. Their nucleoli are fragmented, and although this nuclear division was not followed in detail, the indications are that it is essentially like that in which the ventral canal nucleus is cut off. *Fig. 61* shows the neck divided once longitudinally. In *fig. 62* the neck cells are densely filled with starch and the amount of protoplasm in the central cell has increased greatly, especially in the lower part. In the occurrence of a single large central vacuole in the archegonium Taxodium resembles the Cupresseae and differs from all other conifers so far studied. At this stage we first notice slightly denser areas in the protoplasm at each end. The upper lies very near the nucleus and is smaller than the lower, which occupies a central position in the accumulated basal protoplasm. The nucleus of the central cell at this stage is very like that of the prothallial cells around it. In *fig. 68* there has already appeared around the archegonial group a distinct layer of sheath or jacket cells. At the basal end of the group the angles between the archegonia are filled by these jacket cells, which are at this point generally larger than on the sides. This jacket is a constant accompaniment of the archegonia in all gymnosperms with the exception of Welwitschia. Arnoldi ('00) reports that in Sequoia the layer is incomplete, only certain cells acquiring the distinctive characteristics of modified jacket cells. I have frequently found in Taxodium a cell within this sheath, which in its poverty of contents was easily to be distinguished from its neighbors, and resembles closely the ordinary prothallial cells around it. By far the larger number of the cells directly adjoining the archegonia, however, are modified in the usual way into the nourishing jacket cells. The nucleus of the central cell, at

the stage in *fig. 62*, is larger than the cells of adjoining tissue, but does not differ from them in structure. There is an abundant peripheral reticulum, staining blue throughout with gentian violet, and a nucleolus of compound structure, such as was found in the initial cell nucleus. In place of the single nucleolus there is frequently present a central group of quite distinct granules (*fig. 63*). All stages can be found between the distinct granules and the single compound structure formed by their fusion. From the nucleolus, or from the separate granules, linin threads extend which place the nucleolar matter in direct connection with the reticulum of the nucleus, and this I believe to be a constant character in nucleoli of chromatin material. That this nucleolus is largely composed of chromatin is shown by its subsequent behavior. As already mentioned, the nucleolar structure is the same in the prothallial cells and jacket cells, with the exception that in these the central or nucleolar collection of chromatin is quite inconstant in amount, the size of the nucleolus varying in proportion as the red-staining granules of the reticulum are more or less abundant.

The number of archegonia in a group varies greatly. *Fig. 64* shows a longitudinal section through a group of at least thirty-four archegonia, ten appearing in a single section. This is a larger number than has been found in any other gymnosperm, with the exception of Sequoia. The number is generally from ten to twenty, but in poorly developed prothallia there may be only a half dozen or less. *Fig. 65* is a cross section of a group of seventeen archegonia.

The neck cell very soon after its formation divides by a longitudinal wall into two cells of about equal size. This division is followed usually by another in each cell at right angles to the first, to form a tier of four cells (*fig. 94*). If the neck of the archegonium is crowded or flattened, the second division may occur in one or both of the two first-formed cells (*fig. 93*). As the archegonium reaches maturity, the nuclei of the neck cells generally divide again, and walls may or may not be formed succeeding this division. The walls when formed are very irregular in position. They are frequently somewhat inclined and

may or may not intersect the outer wall of the cell. In this way outer and inner cells are frequently formed (*fig. 66*), but two distinct tiers extending across the neck are never present. *Figs. 66, 93, 96* show the diversity that may occur in the neck. The cells are of unequal size and the number may vary from two to sixteen or even more. Very soon after their last division the neck cells begin to disorganize. They contain starch until the beginning of their disorganization, which takes place somewhat sooner here than in the jacket cells. *Fig. 66* shows an archegonium in which this disorganization is proceeding. There are present at the tip of the protoplasm of the central cell a number of bodies staining deep red with safranin, which may easily be mistaken for fragments of a disorganizing ventral canal nucleus. The last division, however, has not occurred in the central cell, and these bodies are probably the transferred remains of the broken-up nuclei of the neck cells. They are the first such bodies to appear in the archegonium, and in this stage are very conspicuous as a cap at its tip. This early transfer of nutrient material from the neck cells is probably explained by the advantage to be gained in having this transfer completed before fertilization has disturbed the relations between neck and central cell.

The jacket cells around the archegonia generally contain two nuclei each at this time (*figs. 67–69*), but this condition may be reached sooner. About the time that the ventral canal nucleus is cut off, the nuclei of the jacket cells begin to disorganize in the way already described in Cycas by Ikeno ('98) and in Ceratozamia by Arnoldi ('00). The network and the nucleoli resolve themselves into a number of deeply staining bodies, which by the disorganization of the nuclear walls come to lie free in the cytoplasm (*figs. 71–74*).

Immediately before fertilization there begin to appear in the cytoplasm of the egg the proteid vacuoles described in species of Abieteae. Such vacuoles are shown in *fig. 75* and have the same structure that has been described in other cases, but they are not very conspicuous or abundant in Taxodium. Arnoldi ('00) believes these vacuoles to originate in some cases from the

nuclei of the sheath cells of the archegonium, and thinks he has traced their entrance through protoplasmic connections in several species of Pinus and in *Abies sibirica*. From his work on Dammara and Cephalotaxus he is inclined to consider their origin as the same in these cases also. While I have not been able to establish the existence of protoplasmic connections between egg and sheath cells, the appearance in *fig. 83* strongly suggests that such connections exist, and the presence of pits in the wall of the archegonium would also imply their occurrence.

The two denser areas already noticed in the very young archegonium (*fig. 62*) have reached in *fig. 63* a size and condition retained until the initial changes which bring on the division into the central canal and egg nuclei. These areas are of dense fibrous material, and are by far the most striking features in the archegonium of Taxodium. They stain much more deeply with orange G than does the surrounding cytoplasm, and from them fibers radiate to the surface of the cell. It will be noticed that the denser part is at the periphery of the mass, but the inner part is also denser than the ordinary cytoplasm of the cell, and the whole is composed of a complex of granular fibers. The upper of these masses is the smaller and lies very near the nucleus. Fibers can be traced passing from the central mass around the nuclear wall, and they seem to be a continuation of the wall itself. That these bodies are of the same nature as the so-called kinoplasmic material, generally most conspicuous at the time of nuclear division, is evident. They show the same structure as the much less developed kinoplasmic areas already mentioned in the megaspore. Such bodies have not heretofore been described as occurring in such perfection in any case with which I am acquainted. Kinoplasmic areas have been mentioned near the nucleus at the time of its division in the archegonium of *Tsuga canadensis* by Murrill ('00), and Ikeno ('98) has figured such areas under the central cell nucleus of Cycas. Chamberlain ('99) gives one case where there are two such bodies near the egg nucleus in *Pinus Laricio*, and Blackman ('98) describes fibers of this nature radiating from the egg nucleus before fertilization. In all these cases, however, the fibrous material is not nearly so

conspicuous as in Taxodium, and in no case has a second kinoplasmic body been noticed in the lower part of the archegonium. Just before the central cell nucleus prepares to divide the kinoplasmic bodies undergo a marked change. They increase greatly in size, the central part becoming thinner, until finally the denser material, which has by this time become confined to a peripheral position, is broken up into more or less separate groups. In the upper end, one of these groups lying nearest the nucleus becomes most conspicuous, while the others become less and less distinct, their fibers finally arranging themselves into the immense aster which radiates from the inner side of the nucleus. Fibers extend around the nucleus from the center of the aster and merge insensibly into the nuclear wall (*fig. 78*). The lower kinoplasmic mass has also become broken up into separate parts, forming an incomplete ring, and during the activities in the upper part connected with the cutting off of the ventral canal nucleus these separated groups below become likewise extremely active and fill the whole base of the archegonium with conspicuous figures of various shapes, such as fans, asters, double asters, test-tube cleaners, etc. In fact the whole kinoplasmic content of the archegonium seems as if electrified. A few of these structures are shown in *figs. 90–92*.

Chamberlain ('99) figures such radiations in the egg of *Pinus Laricio* after the formation of the ventral canal nucleus, and considers them as parts of the enormously developed spindle of that division. In Taxodium, however, the kinoplasmic radiations in the lower part of the archegonium are in no way connected with the spindle above, although they are most active at the time of its formation. The function of these kinoplasmic masses is obscure, and I can suggest no explanation unless it be that the great length of the archegonia in Taxodium and the fact that it is principally at the end farthest away from the nucleus that they are exposed to the sheath cells may make it advantageous to have a more definite mechanism for the regulation of the entrance of the plastic material at this end.

[*To be concluded.*]

ON THE GAMETOPHYTES AND EMBRYO OF TAXODIUM.

CONTRIBUTIONS FROM THE BOTANICAL LABORATORY OF THE JOHNS HOPKINS UNIVERSITY, No. 1.

W. C. Coker.

(WITH PLATES I–XI)

(*Concluded from p. 27*)

FORMATION OF VENTRAL CANAL NUCLEUS.

Murrill ('00) has recently described in Tsuga a peculiar method of spindle-formation in the division of the central cell of the archegonium. He finds a kinoplasmic area in contact with the inner side of the nucleus just before its division. Fibers begin to grow from this area into the nucleus, pressing before them the nuclear wall. They gradually extend until they come to occupy the greater part of the nuclear cavity, and are then joined by similar fibers which have come in from the opposite pole to form the prophase of the spindle. These observations I cannot confirm in Taxodium. *Figs. 76–90* show this division in detail. As already stated, fibers pass around the nucleus from the kinoplasmic areas or aster and merge insensibly into the nuclear wall. The nuclear wall is frequently considerably drawn in at a point opposite the kinoplasmic body (*figs. 76, 78*), but no strong fibers can be seen extending from one to the other at this point. In fact, this collapse is generally more noticeable in the early stages of preparatory changes. Murrill ('01) finds such fibers, which he concludes have caused the depression of the nuclear wall.

The arrangement between aster and nucleus at this time may be best compared to that between the car of a balloon and the bag above it, the ensheathing fibers of the nucleus terminating in the aster representing the ropes around the balloon connecting it with the car below. *Fig. 76* shows the beginning of the changes in the nucleus which lead to the formation of the spindle.

Synapsis has occurred, and the nuclear reticulum forms a dense tangle near the nucleolus. The latter can now be distinctly seen to be of compound structure, and from it threads can be traced into the network. Fine threads also connect the conspicuous central tangle with the nuclear walls. Such a synapsis stage is described by Murrill in the nucleus of the central cell of Tsuga. In *fig. 72* the reticulum has begun to move back into its original position. The nucleolus is now distinctly fragmented into a number of granules of apparently equal size, which gradually become more and more separated into a broken ring or coiled thread (*figs. 78–80*). These granules retain the deep red stain characteristic of the nucleolus in previous stages. The ring, or thread, is now broken up more and more into separate parts and distributed near the periphery of the nucleus (*figs. 81–84*) and the reticulum of the nucleus begins to arrange itself for the formation of the spindle (*figs. 83–85*). The fibrous connections between the reticulum and the nucleolar thread are to be noticed at all stages of its distribution.

The red-staining granules derived from the nucleolus have not lost their identity at any stage. They become gradually elongated and thin (*figs. 85, 86*), and thus approach more and more the characteristic structure of the chromosomes. The fibers of the reticulum draw together at different points of the nucleus and certain centrally placed ones become distinguishable as spindle fibers, while the mantle threads pass gradually into the unmodified reticulum of the nucleus. The granular nature of the spindle is evident even at so late a stage as *fig. 86*. The nuclear wall has meanwhile disappeared, but its position is indicated by the arrangement of the fibers until the anaphase stage of division (*fig. 87*). The chromosomes, as they are drawn apart, are of the usual V or U shape (*fig. 87*). The transformation of the nucleolus into the chromosomes may be followed without interruption through its whole course, not only by reactions in staining, but in serial development.

Chromatin nucleoli have frequently been described, but principally among the lower plants. Strasburger ('00) in his recent work devotes considerable space to the discussion of the part

played by the nucleolus in the formation of the spindle. He reviews the present knowledge of the subject, and while acknowledging the chromatin character of the nucleolus in a few cases, expresses himself as strongly inclined toward the view that in the higher plants at least it is of kinoplasmic or spindle-building material. Nucleoli of chromatin character have been described in Corallina by Davis ('98), in Spirogyra by Mitzkewitsch ('85), in Bignonia by Duggar ('99), and in a number of other plants. That the nucleolus of the central cell in Taxodium is directly transformed into chromosomes seems to be evident from the figures given, and I think we may be equally sure of the intranuclear origin of the spindle. The kinoplasmic mass at the tip of the spindle seems to serve merely as a point of attachment for the spindle fibers, its numerous radiations making a firm foundation for the orientation of the spindle, as suggested by Murrill ('01).

The nucleus of the central cell, just before its division, is generally situated not far from the tip of the archegonium, but it is often found as much as a third of the way down. In such cases the central vacuole, which is now much smaller, lies nearer the base of the archegonium. The wall of the nucleus is at the very surface of the cell (*figs. 66, 78*), and it is often impossible to distinguish any protoplasm between nucleus and surface. In *figs. 88* and *89* there is to be noticed in the center of the spindle a broad zone which is composed of numerous granules. In Flemming's triple this zone stains a deeper violet than the other parts of the spindle, and occupies the position at which we might expect to find a cell plate. No cell plate is formed, however, and the whole spindle fades away into the cytoplasm of the cell. At the surface end of the spindle shown in *fig. 87* a small aster has appeared. The daughter nuclei are formed in the centers of these asters (*fig. 88*), and as they increase in size the kinoplasm is collected more and more on the sides toward the spindle (*figs. 89, 90*). In this way the egg and ventral canal nuclei are both furnished with a tuft of kinoplasm on their inner faces. Other tufts may be located at different positions around the egg nucleus (*fig. 90*), but in

later stages the egg nucleus is nearly always furnished with but a single tuft on the side toward the base of the archegonium. The spindle may be situated in every position in reference to the axis of the archegonium, parallel (*fig. 87*), perpendicular (*fig. 88*), or inclined (*figs. 86, 89*), but the inner pole is always located in the center of the aster. The spindle may extend either up or down from the inner pole, and the ventral canal nucleus may thus be placed either above or below the egg nucleus (*fig. 91*). The archegonium to the right in *fig. 92* shows a ventral canal nucleus situated nearer the base than the tip, and in the archegonium to the left the ventral canal nucleus occupies a nearly central position, the egg nucleus lying even lower. It is only rarely that the ventral canal nucleus is situated at the very tip of the egg. Such a position is shown in *figs. 87* and *90*.

This formation of the ventral canal nucleus in any region other than near the tip of the archegonium has not been described in any other gymnosperm so far as I am aware. This peculiarity in Taxodium seems to be an acquired character for the protection of the ventral canal nucleus during fertilization, for, as we are to see later, it is to play a part in the subsequent history of the egg. In most gymnosperms the ventral canal nucleus is furnished with a certain amount of protoplasm of its own which is distinctly separate from that of the egg. Arnoldi ('00) lays stress on what he calls the absence of a ventral canal cell in Cephalotaxus. He considers the ventral canal cell lacking because no wall is formed between the ventral canal nucleus and the egg. In this case, however, a certain amount of protoplasm becomes disorganized around the ventral canal nucleus, and swelling up is said to burst open the neck of the archegonium. If this is the case, the disorganizing protoplasm may be considered as belonging to the ventral canal nucleus, and a ventral canal cell could not strictly be said to be absent.

In Taxodium, however, not even a cell plate is formed, and the ventral canal nucleus lies perfectly free in the cytoplasm of the egg without a distinct protoplasmic area of its own. A ventral canal cell in the strict sense is therefore lacking here.

From Strasburger's ('79) account it seems probable that this is equally true in the Cupresseae. Ikeno ('01) has well remarked that the important point is the division of the nucleus and not the separation of a small amount of protoplasm from the egg. Arnold ('99) denies the occurrence of even a ventral canal nucleus in Sequoia.

The ventral canal nucleus remains closely applied to the surface of the egg and is somewhat flattened or compressed. It does not go through the peculiar changes which occur in the egg nucleus before fertilization, but having reached the condition shown in *fig. 90* does not develop much further until after the fertilization of the egg. It is furnished with a chromatin reticulum and an evident nucleolus. The ventral canal nucleus has usually been described as undergoing disorganization soon after its formation, sometimes reaching a resting condition, but generally never developing further than the first stages of this condition (Blackman, '98; Ikeno, '98; Murrill, '01, among others). Chamberlain ('98) figures a well-developed nucleus in the young fertilized archegonium of *Pinus Laricio*, which closely resembled the egg nucleus and which is, as he suggests, in all probability the ventral canal nucleus. Very recently Ikeno ('01) has described a large nucleus in the tip of the egg of Ginkgo and called it a ventral canal nucleus. He also calls attention to the possibility that a persistent ventral canal nucleus may have been mistaken for the extra male nucleus among previous observers. It has also probably been figured as the functional male nucleus.

The position of the ventral canal nucleus in Taxodium at some protected place on the side of the archegonium has preserved it from destruction during the entrance of the sperm cell, and as the fusing male and female nuclei approach the base of the archegonium the ventral canal nucleus begins to extend into the center, and by amitotic division it usually gives rise to the several nuclei of different sizes which occupy the upper half of the egg (*figs. 107, 112*). If the egg is not fertilized, the ventral canal nucleus generally remains unchanged in its original position (*fig. 107*), but it not infrequently increases in size and moves

nearer the center of the egg at about the time that those of the fertilized archegonia are dividing amitotically. In these cases it does not divide, but increasing greatly in size comes to resemble the egg nucleus just as in the case described by Chamberlain ('98) (*fig. 114*). Indications of amitotic division may be sometimes seen in the ventral canal nucleus in unfertilized archegonia (*fig. 113*), but such cases are rare. The development of the ventral canal nucleus in fertilized archegonia is not the exception, but the rule. In almost all cases in which there is a proembryo present there are to be found in the upper part of the archegonium a varying number of nuclei of different sizes which have been derived from the ventral canal nucleus (*figs. 115, 116*). In some cases, as would naturally follow when the ventral canal cell is cut off far down the archegonium, the nuclei derived from it may occupy a position near the base, and consequently near the proembryo. *Fig. 118* is such a case. Here, the ventral canal nuclei are sharply distinguished from the nuclei of the embryo (only one of which is shown) by the entire absence of the starch sheath characteristic of the latter. The ventral canal nuclei are usually separated from the proembryo by a distinct area of disorganizing protoplasm, but they themselves seem to retain a part of the protoplasm at the tip of the archegonium which is not disorganized until much later (*fig. 116*).

DEVELOPMENT OF THE FEMALE NUCLEUS.

The female nucleus in its very young stages is shown in *figs. 88* and *89*. It develops more rapidly than the ventral canal nucleus and goes through the peculiar changes of structure which have already been described in more or less detail by others. The chromosomes spin themselves out into a reticulum which is apparently often arranged in a spiral form (*fig. 89*). A nucleolus begins to appear early. At the stage of *fig. 89* the nucleus is not yet furnished with a distinct membrane, but in *fig. 90* the wall has appeared. There is first seen at this stage a finely granular substance which has been called metaplasmic substance by Strasburger and some recent workers. From the subsequent behavior of this substance, it would be better described

as kinoplasm than as metaplasm, but as the former word is overworked I shall follow Chamberlain ('99) and speak of it as linin, from which it does not seem to differ. It is at first entirely distinct from the reticulum, and is only very slightly stained in Flemming's triple. The nucleolus increases in size and the linin granules become more abundant as the nucleus develops. The reticulum still remains perfectly distinct from the granular substance and stains a bright red in safranin. The nucleolus is of quite a different character from the nucleolus of the central cell previously described. It becomes larger and larger until, at the time of fertilization, it is very conspicuous (*fig.* 100). When deeply stained, it seems to be entirely homogeneous, but on washing out a fine alveolar structure is to be seen. This does not in the least recall the irregular compound nucleolus of the central cell, and from the entirely different behavior of these two nucleoli during cell division it is evident they are of a different nature. The large nucleolus of the egg nucleus may be called the plastin nucleolus, while that of the central cell is a chromatin nucleolus. The plastin nucleolus of the egg nucleus seems to loose its stain more easily at one time than at another, as it is frequently found almost colorless. This loss of staining quality may be due to fluctuations in the amount of plastin material inclosed in its honey-comb-like structure during the active stages of development. This seems more probable, as it is often found when unstained in a collapsed condition, a distinct but thin shell being always present.

At the time of fertilization the female nucleus may vary considerably in its structure. Sometimes the linin material remains finely granular up to the time of fertilization. In such cases it is easily distinguishable from the chromatin reticulum, which on account of the huge size of the nucleus is now relatively scarce. The large plastin nucleolus is always present and is not connected in any way with the reticulum. This is another character which distinguishes it from the chromatin nucleoli already described. It is more frequently the case that the linin substance of the nucleolus, before the entrance of the male nucleus, has become grouped into an abundant reticulum, upon which granules of

various sizes are deposited (*fig. 101*). The larger of these linin granules take the red stain, and thus become indistinguishable from the chromatin masses of the nucleus. At no stage in the development of the egg nucleus is it without a distinct reticulum. Whether the chromatin reticulum contracts to form separate chromatin masses or whether it remains as the coarser part of the reticulum shown in *figs. 100* and *101* seems uncertain.

Chamberlain ('99) and Blackman ('98) have minutely described the development of the female nucleus in *Pinus Laricio* and *Pinus sylvestris*, respectively. The former describes the complete disappearance of the nuclear reticulum at two stages of the development of the nucleus. At the time of fertilization the chromatin is all grouped in the center of the egg nucleus, the remainder being occupied by the finely granular linin substance. This condition would not be so different from the case in Taxodium if the chromatin reticulum of the latter should resolve itself into chromatin nucleoli before the organization of the linin substance into a distinct reticulum. Such a condition, however, has not been found. In the mature egg nucleus the number of small nucleoli which take the red stain with safranin is sometimes no larger than could be supplied by the chromatin of the nucleus, but when the linin substance becomes collected into large granules, which is not at all unusual, they also take the red stain and cannot easily be distinguished from the chromatin nucleoli. Their color, however, is usually a more purplish red than is the case with the chromatin nucleoli, and if the washing is continued they are the first to lose the reddish stain.

FERTILIZATION.

We left the pollen tube after describing the formation of the male cells. The divisions of the central cell of the archegonium and of the central cell of the pollen tube occur simultaneously, or almost so, in every case observed, and in this respect again resemble the Cupresseae (Strasburger, '79). Fertilization occurs in a very short time after the completion of these divisions. As stated in my previous note, both sperm cells may enter one archegonium, but this is by no means always the case, in fact

not generally so; and when it does occur it may be considered as a failure to secure the results aimed at. When several pollen tubes have reached the archegonial group (as many as five have been noticed) the entrance of two or more sperm cells into an archegonium is not unusual, but when the number of tubes is only one or two it less often occurs. Pollen tubes sometimes are so abundant that all cannot pass into the depression above the archegonial group. Some then remain in different positions around the tip of the prothallium, and are often to be seen unchanged after their more fortunate neighbors have fertilized the archegonia. The central cell of such pollen tubes often divides at the same time with those over the archegonia, but in some cases where they are small and badly nourished the central cell does not divide at all.

In addition to the one or two sperm nuclei that enter the archegonium the disorganized remains of the stalk and tube nucleus with their surrounding protoplasm are also swept in, along with what is left of the neck cells. Prothallial nuclei are also sometimes thrown in, and quite a collection of such heterogeneous material may often be seen in the archegonial tip above the protoplasm of the egg. Most observers who have described the discharge of extra sperm nuclei or vegetative cells into the fertilized archegonium have made no distinction between entrance into archegonium and entrance into egg. Webber ('97) says that several spermatozoids may enter a single archegonium in Zamia, but that only the one that is used in fertilization actually enters the cytoplasm of the egg, the others remaining above and free in the cavity of the archegonial tip. Ikeno ('01) calls attention to this point, and finds that in Ginkgo the supernumerary spermatozoids do not enter the cytoplasm of the egg, but remain distinct from it, even if actually pressed into its surface. He also mentions the possibility that certain nuclei in the tip of the archegonia, that have in previous cases been described as derived from the sperm cell, are probably the results of amitotic division of a persistent ventral canal cell. These observations of Webber and of Ikeno I can positively confirm in Taxodium. The supernumerary sperm cells remain separate from the cytoplasm of the

egg, and may be long distinguished as they slowly disorganize (*figs. 104, 110*).

The functional sperm cell in its course to the egg nucleus is shown in *figs. 100* and *101*. Its protoplasm becomes intimately connected with that of the egg, but its identity is not lost at any stage. The starch sheath still remains in close contact with the sperm nucleus, and as its boundary touches the wall of the female nucleus it spreads around it more and more, while the sperm nucleus in its center finally comes in contact with the nucleus of the egg (*fig. 102*). This starch sheath of the sperm nucleus furnishes the sheath which is characteristic of the fusion nucleus.

Strasburger has several times spoken of the sudden appearance of starch in the fertilized egg of the Cupresseae. In his *Neue Untersuchungen* (1884) he expresses surprise at this, because, as he says, the pollen tube here contains very little starch before fertilization. His description in Juniperus of the starch sheath appearing around the fusion nucleus immediately after its formation and the subsequent behavior of the two may be applied almost word for word to Taxodium. It seems probable, therefore, that starch may yet be found in the male cells of the Cupresseae.

The sperm nucleus before contact with the female has the same character as was noticed in the pollen tube. It is difficult to distinguish it from the protoplasm around it, but a central nucleolus and a scarce reticulum may be made out in the dense substance that completely fills the nucleus. When the sperm cell enters the egg its nucleus is not more than a fifth the diameter of the egg nucleus (*fig. 100*), but during its course to the latter the sperm nucleus enlarges somewhat (*fig. 101*), and just after contact the diameter of the two nuclei is about as one to two. The male protoplasm, with its inclosed starch, now completely infolds the egg nucleus until it becomes evenly distributed around it (*figs. 102, 103, 105*). The structure of the sperm nucleus begins to change immediately on contact with the egg nucleus. The linin substance becomes arranged into a reticulum staining deep blue with gentian, while the nucleolus seems to break up into a number of smaller granules which lie more or

less grouped, but frequently scattered in no definite position. They were not found to be definitely located near the point of contact between the two nuclei as described by Blackman ('98) and Chamberlain ('99). The fusion nucleus begins to move to the base of the archegonium, and its male and female elements can be easily distinguished until the base is nearly reached (*fig. 106*). This passing of the fusion nucleus to the base of the archegonium before division occurs has been described by Strasburger ('79, '84) in Juniperus and by Jäger ('00) in Taxus.

The partition between the two nuclei does not entirely disappear until immediately before the first division. *Fig. 119* represents the two nuclei just after the disappearance of the separating wall. The parts derived from each are still distinct, the denser part being the male. The reticulum of the sperm nucleus is arranged in a more or less radiating way, and that of the egg is also becoming thus arranged. The large plastin nucleolus of the egg nucleus may be found in all stages of fusion. In addition to the reticulum and plastin nucleolus there are also present numbers of chromatin nucleoli in each half of the nucleus. The spindle of the first division is derived entirely from the reticulum of the fusion nucleus.

The structure of the male or sperm cell of Taxodium and its behavior during fertilization are worthy of especial emphasis. The presence of large quantities of starch around its nucleus and the transfer of this starch, together with its protoplasm, to form a distinct layer around the egg nucleus, which later becomes separated from the protoplasm of the egg in the base of the archegonium to form the greater part of the young embryo, are peculiarities not as yet described in any other organism, and they seem of sufficient interest to receive attention in any comparative study of sexual cells.

All observers who have studied fertilization in the gymnosperms seem agreed that the male nucleus slips from its protoplasmic sheath as it approaches the egg nucleus and leaves it behind near the point of entrance. If the case is as they think, Taxodium is an exception here.

In his well-known work on chromatophores, leucoplasts, etc.,

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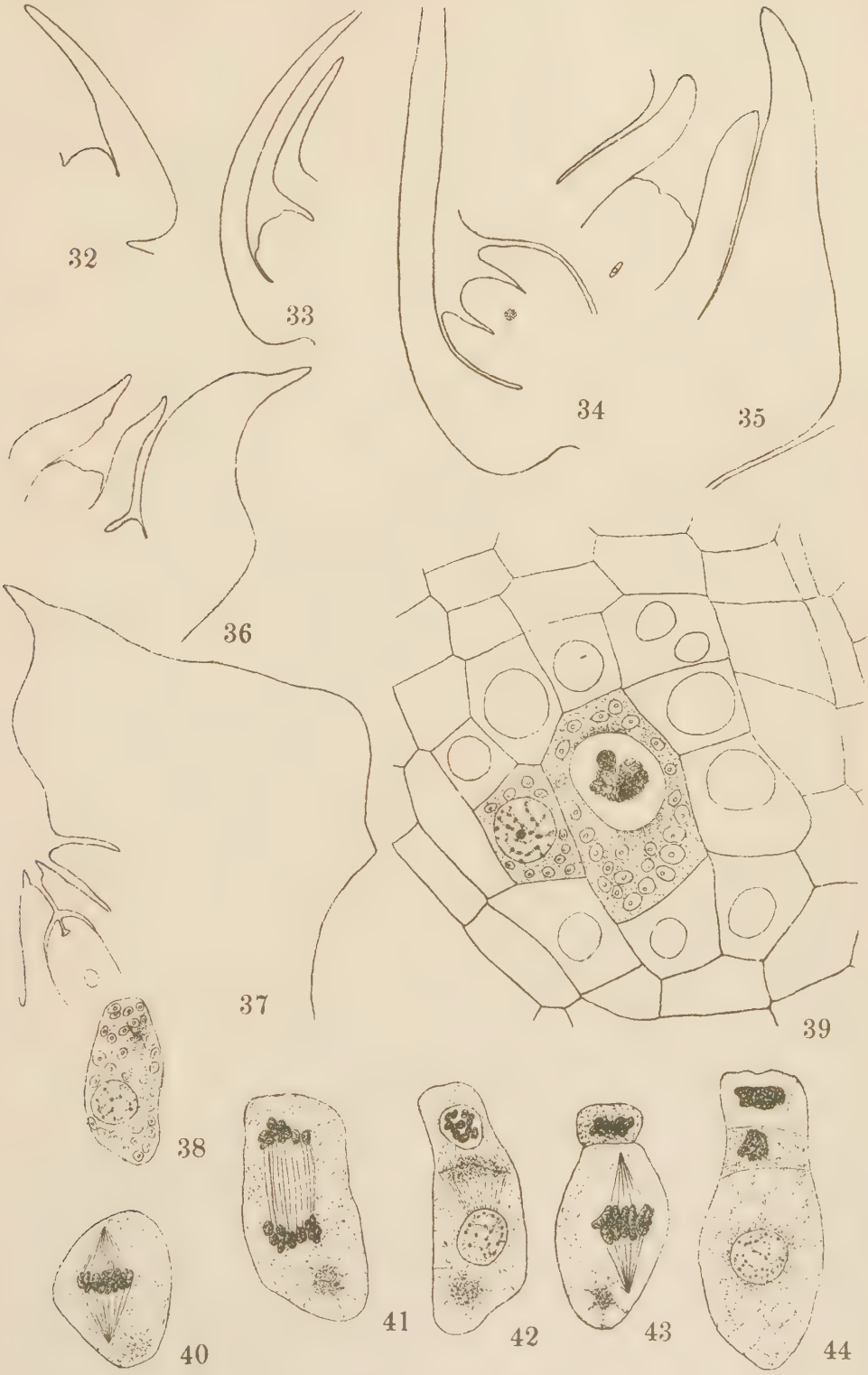

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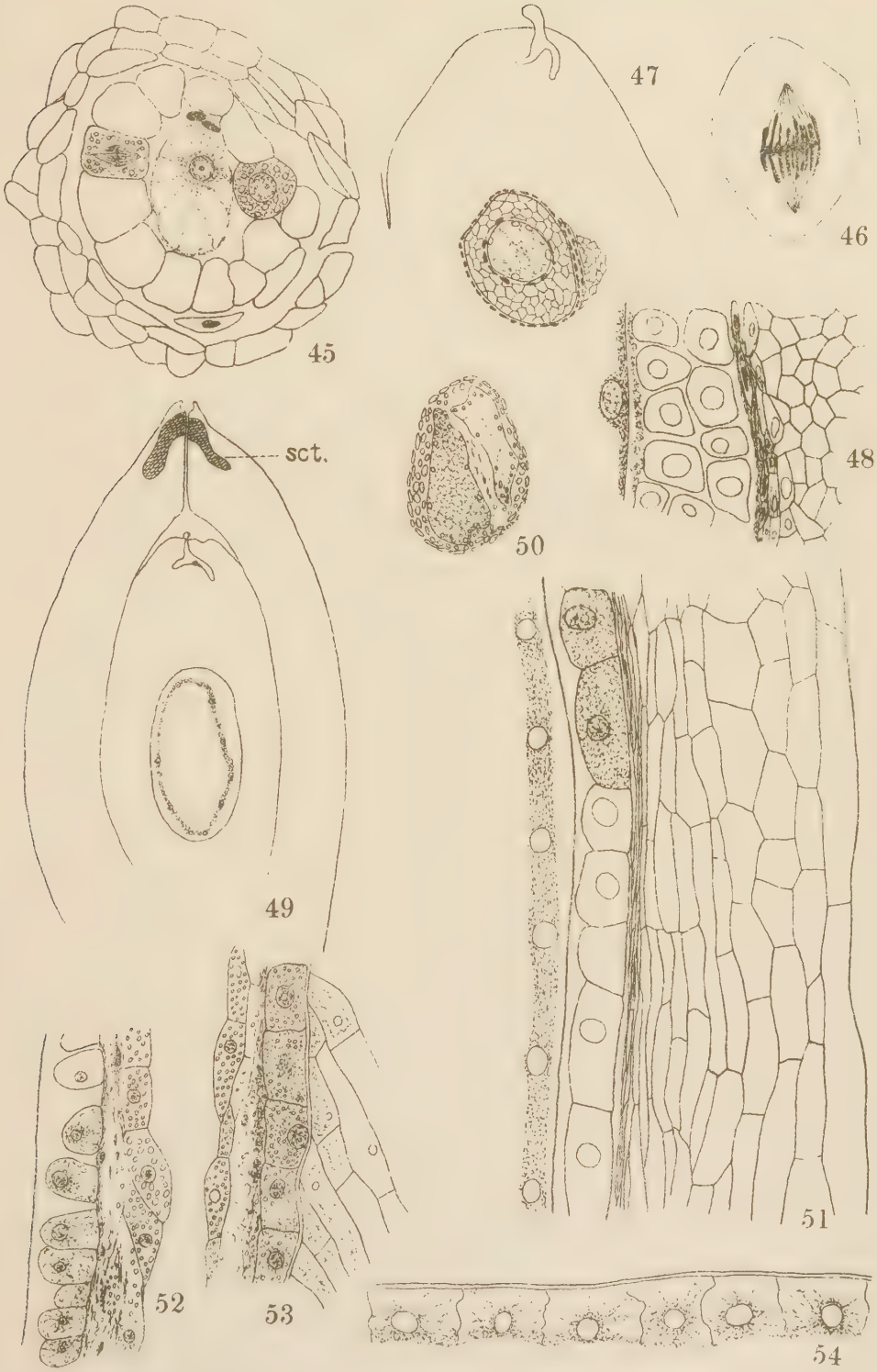

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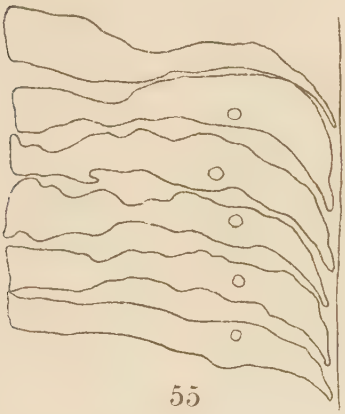

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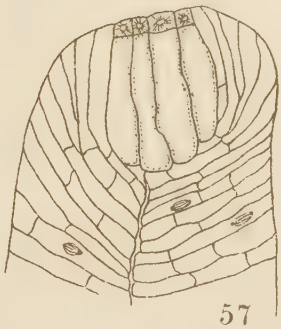

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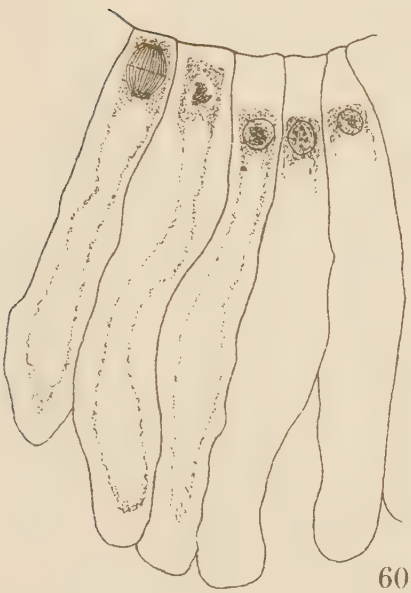

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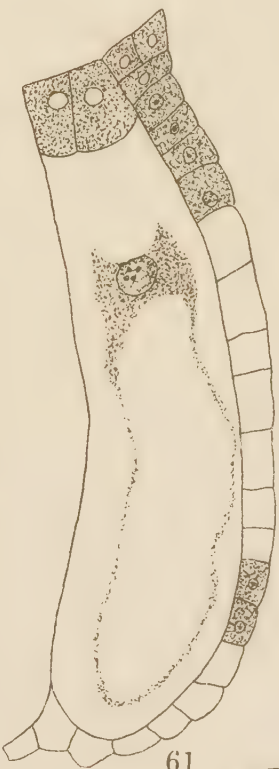

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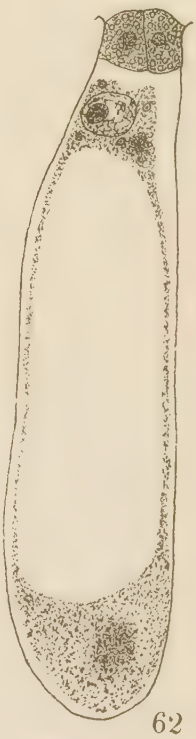

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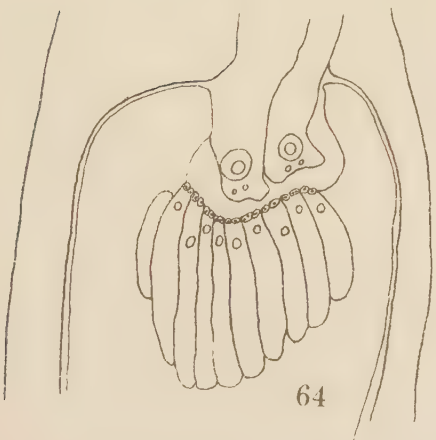

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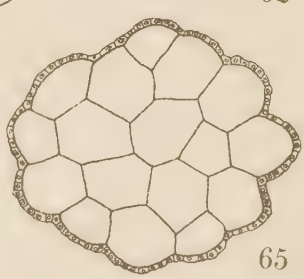

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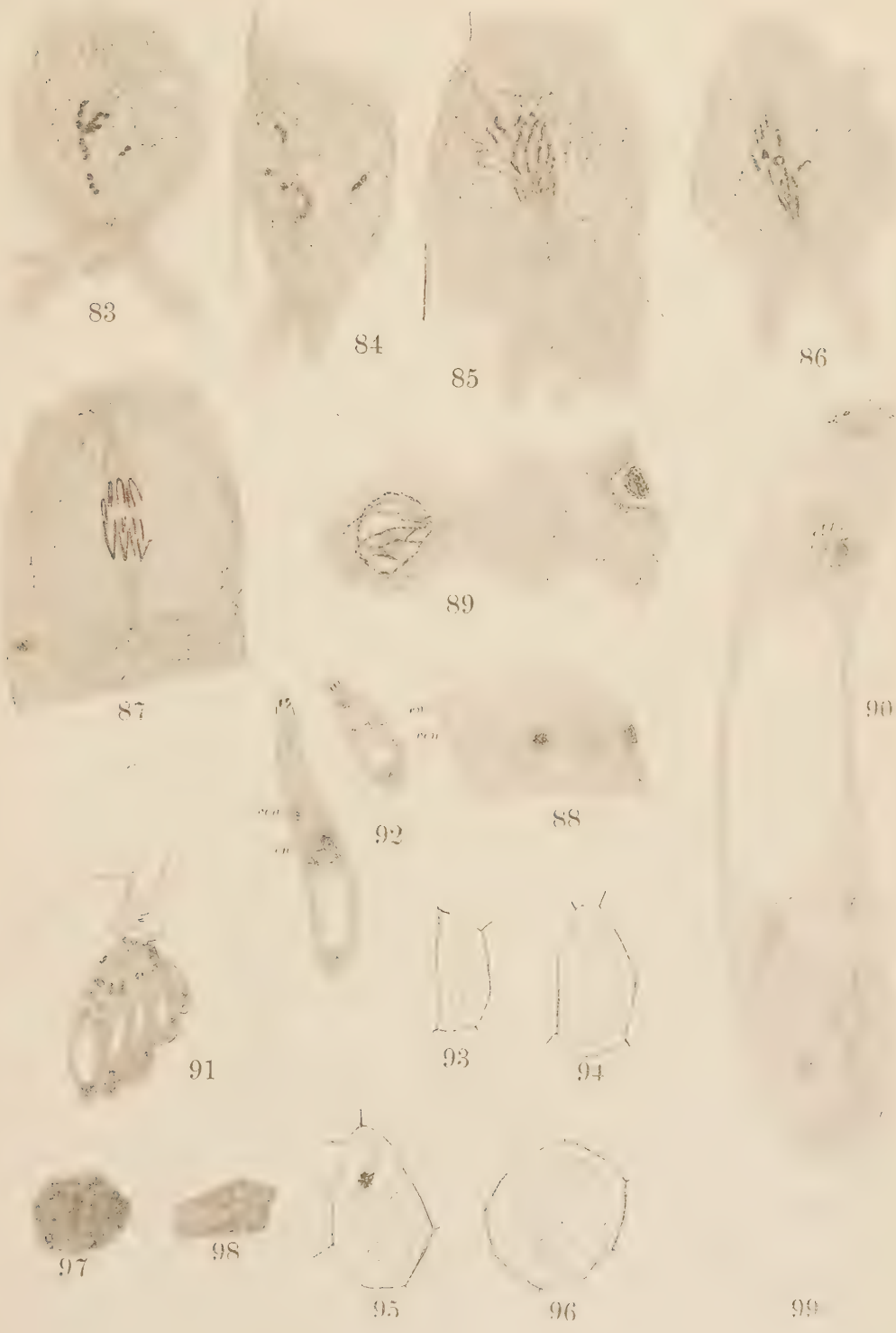

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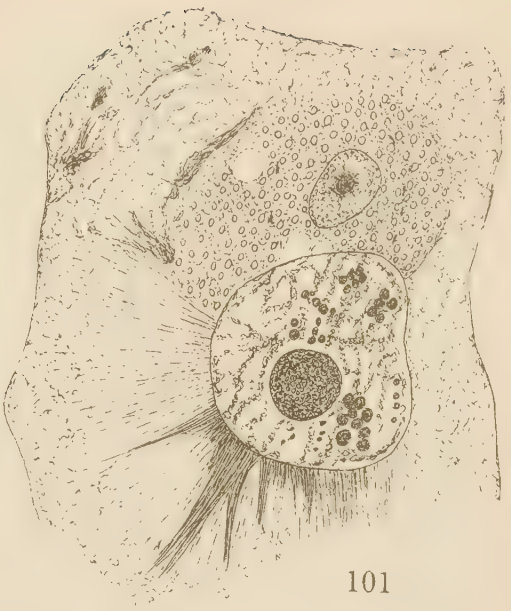

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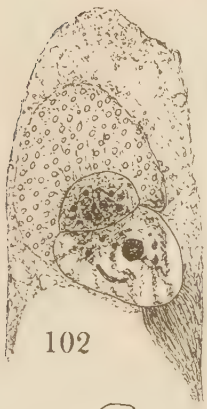

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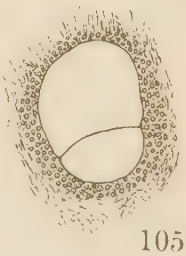

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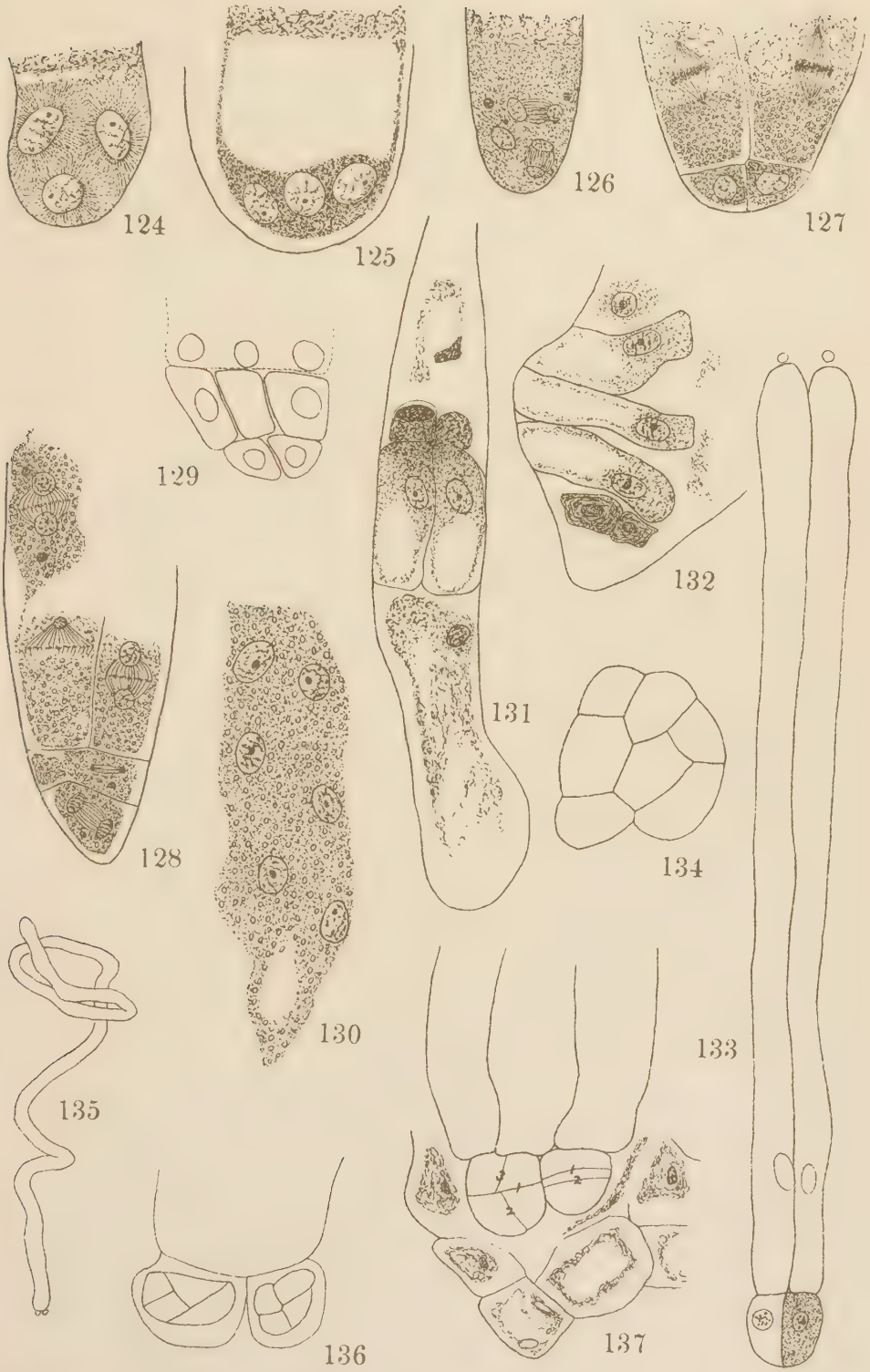

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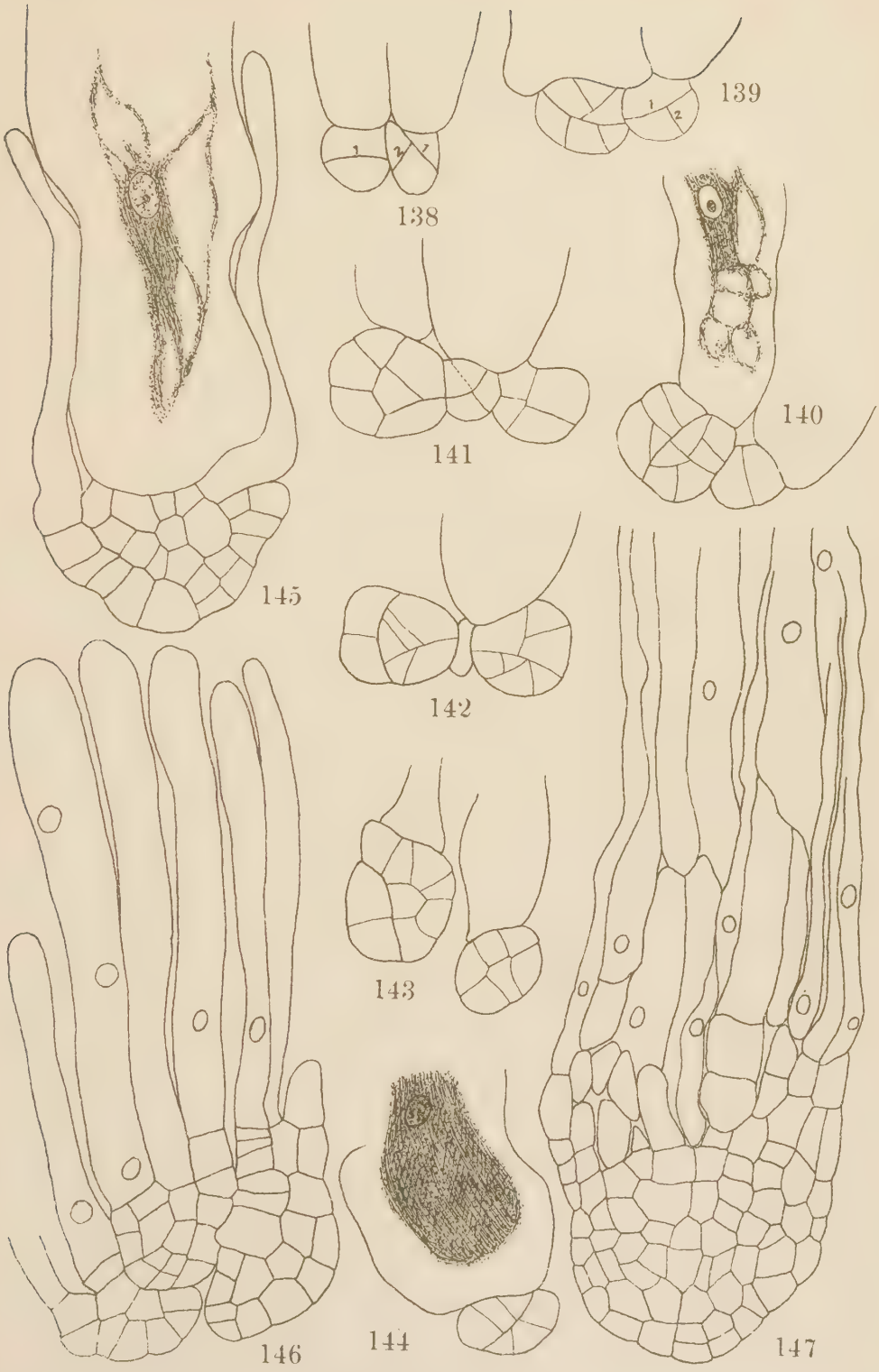

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A. F. W. Schimper demonstrates the presence of plastids in the egg cells of plants. Taking the occurrence of starch as an indication of the presence of leucoplasts, we find that most of the plastids of the proembryo of Taxodium are furnished by the male cell. Occasionally a few scattered starch grains may be seen in the archegonium before fertilization, but as a rule the egg protoplasm is entirely free from them at maturity. Chromatophores or leucoplasts might of course be present in the egg without the occurrence of starch, and as such bodies are rather difficult to demonstrate without special methods, their presence cannot be denied. Blackman's ('98) observation of leucoplasts in the male cell of Pinus has already been referred to.

THE EMBRYO.

Fig. 120 shows an early stage in the formation of the spindle of the first division. The reticulum has become less coarse, the larger granules seeming to become transformed into smaller ones which characterize the fibers of the spindle at this stage. The outlines of the fusion nucleus are still quite distinct, and its fibers pass into those of the spindle imperceptibly. The chromatin has become arranged on the more homogeneous fibers in the center, and form ill-defined, much-elongated bodies which are not yet grouped into a definite plate. It will be seen that the spindle is multipolar at its origin. Stages of older spindles were not found.

The plastin nucleolus derived from the egg nucleus does not disappear during the spindle formation or during division of the nucleus. It is generally inclosed in one of the daughter nuclei, but is sometimes left free in the surrounding cytoplasm. The two daughter nuclei of the first division are first separated a considerable distance (*fig. 121*), but later approach nearer and are surrounded by the same starch sheath (*fig. 117*). Their structure is very similar to that of the egg nucleus before fertilization, a plastin nucleus, a chromatin reticulum, and a large amount of finely granular linin material being present. As the fusion nucleus approaches the base of the archegonium, it may rotate so that the part derived from the sperm nucleus lies nearest the base of the archegonium (*fig. 106*). The large vacuole of the

egg becomes broken up into smaller ones as the fusion nucleus approaches it, and in some cases almost disappears; often, however, the distinct starch-containing protoplasmic sheath of the fusion nucleus or its daughter nuclei is surrounded on both sides by large vacuoles (*fig. 117*).

The two daughter nuclei of the first division very soon prepare for the second division. *Fig. 122* shows such a nucleus in an early stage of mitosis. The chromatin is arranged in distinct rods, the linin granules have formed the reticulum, and the large plastin nucleolus is conspicuous. *Fig. 123* represents a late anaphase in the spindle of the second division. The number of chromosomes is evidently greater than in the division of the central cell. At this time the distinctive protoplasm of the proembryo has filled the base of the archegonium and is becoming more and more distinct from the ordinary egg cytoplasm above it. During this division and the one following, the plastin nucleolus is frequently left outside of the daughter nuclei, and may be broken up into several parts which lie free in the cytoplasm. *Fig. 124* gives the usual arrangement of the first four nuclei of the proembryo. They lie tetrad-fashion in the base of the archegonium, and a narrow zone of disorganizing protoplasm is beginning to appear above the starch-containing sheath around them. The cytoplasm is arranged in radiating lines from the nuclei. In *fig. 126* the third division has taken place. Several plastin nucleoli are present in the cytoplasm. In *fig. 125* the four nuclei of the second division are arranged in an unusual manner. They lie in one plane at the base of the archegonium and are bounded above by a large vacuole.

Spindles of the third division do not have any definite arrangement in reference to each other or to the axis of the archegonium, but their position seems to depend a good deal upon the width of the archegonium. After the formation of the daughter nuclei, however, these become arranged as *fig. 116*. Two are situated side by side at the base, and six lie above them in one plane. While this is the usual arrangement, it is not uncommon to find only one at the base, while the other seven are arranged above it. In a few cases there were three below

and five above. An abnormal condition is shown in *fig. 128*. Here two of the eight nuclei produced by the third division have not joined in the formation of the basal group, but remain some distance above, quite separate from the proembryo beneath. Two nuclei are at the base in this case, as usual, but the number above is only four, thus making up the total eight. All these nuclei are in division. The section is somewhat oblique, so that the base of one of the upper cells is shown above the two basal cells.

After the cells are arranged as mentioned, they become separated from each other by delicate cell walls, the upper tier remaining open at the top. The fourth division now takes place almost simultaneously in all of the nuclei. The axes of the spindles in the upper tier are parallel with the axis of the archegonium (*fig. 127*). There is thus cut off from the upper cells an equal number of nuclei which lie free in the cytoplasm of the egg and form the rosette (*fig. 129*). Cell walls are produced by this division and the middle tier is now completely closed (*fig. 129*). By referring to *fig. 127* it will be seen that the starch is all grouped in the base ends of the upper tier of open cells, and that the lower poles of the spindles are imbedded in it. When walls are formed all the starch is inclosed in the middle tier with the exception of a few scattered grains in the tip cells. The spindles in the lower cells lie at right angles to the ones above, and form a tier of four cells, or of six in those cases where three original cells have been arranged at the tip, or only two where there is but one tip cell. The middle tier now elongates into the suspensors (*fig. 133*), the nuclei and most of the cytoplasm appearing at the lower end. Further division in the tip cells does not occur until the suspensors have greatly elongated.

The formation of the embryo proper shows much variation. The suspensors from a single archegonium only rarely remain completely united at their tips, but usually separate more or less. The supernumerary suspensors are left behind at various positions, so that at the time of the first division of the tip cells the number of suspensors and tip cells is usually the

same. Each tip cell divides independently of the others, even when they are near together, and different stages of development are found in embryos from the same archegonium. Generally the tip cells are separated by the spreading or unequal growth of the suspensors. *Fig. 143* shows two embryos from the same archegonium, each with its own suspensor. In *fig. 144* the embryo and suspensor shown are situated at a considerable distance from any others.

Figs. 140–142 are serial sections through a group of embryos from one archegonium. There are four in the group, only three of which are shown. It will be seen that the growth in each proceeds independently, and some of them are more advanced than others. In *fig. 139* a two-celled and a three-celled embryo are represented. The first wall is almost always inclined and produces two cells generally of unequal size. The second wall arises in the distal larger cell, and is nearly at right angles to the first. The following divisions cannot be systematized by any rule, but, as will appear from the figures, are irregularly disposed. *Fig. 135* shows a suspensor bearing two embryos on its tip. It was teased from a prothallium and mounted whole. *Fig. 136* is a more magnified view of its tip; the two embryos are proceeding each on its own course in spite of the close contact of their original walls. In *fig. 146* the more advanced embryo seems to be formed of three distinct parts which may be interpreted as derived from three separate tip cells which proceeded alone in their development for a time, but being closely associated have united to form a single embryo. In *fig. 145* a single suspensor bears a single embryo. The embryonal tubes have appeared, but are not quite so much developed as in *fig. 146*. In *fig. 147* is shown the most advanced stage obtained of the young embryo. The embryonal tubes are very numerous and extend about four times farther up than is shown in the figure. They have completely filled the space previously occupied by the suspensor, of which no trace can be seen at this time. Three abnormal embryos are shown in *figs. 130–132*.

From this description it will be seen that the development of the embryo of Taxodium differs from all other conifers. In

Taxus (Jäger, '99) and Cephalotaxus (Arnoldi, '00) there are more than eight free nuclei present before cell formation, and the tiers are not so definite as in Taxodium. In the Cupresseae (Strasburger, '72) the tiers are at first composed of single cells, while in the Abieteae only one tier is first formed which then gives rise to four by successive divisions. Coulter ('97) and Coulter and Chamberlain ('01) have described peculiarities in the number of embryos formed from one archegonium, and in the relation of suspensors to embryos in *Pinus Laricio*, which are not unlike the diversities found in Taxodium.

SYSTEMATIC POSITION OF TAXODIUM.

The family Taxodieae as arranged by Eichler is acknowledged a tentative one, and when we compare the gametophytes of Sequoia and Taxodium, the only two genera of this family in which this part of the life history has been followed, we are impressed, not with similarities, but with divergencies; and it becomes at once apparent that, if gametophytic characters are of any consequence in classification, the group as it now stands is an artificial one and must be rearranged. The points of divergence between Taxodium and Sequoia are so striking that to retain them longer in the same group would do violence to taxonomic principles. In the large number of functional megaspores and prothallia present in its sporangia Sequoia is markedly primitive, and in the arrangement and number of its archegonia it is, so far as known, *sui generis*. Its male gametophyte is imperfectly known, the young stages being quite unstudied.

In the preceding pages attention has been called in passing to certain of the more evident structural similarities between Taxodium and the Cupresseae, and for the sake of brevity they will not again be rehearsed here. It is sufficient to say that Taxodium agrees with the Cupresseae in all gametophytic characters that seem to me of much taxonomic importance, and I think it evident that such essential agreement with the Cupresseae on the one hand and such fundamental divergence from Sequoia on the other must necessitate the removal of Taxodium from its connection with the latter and its insertion in the former family.

Such a change will require us to discard the name Taxodieae and combine the remaining genera of the family into some tentative group of another name until further study shall make their position clear.

SUMMARY.

The staminate cones begin to develop in September or October, and by winter the pollen mother-cells are formed. In spring starch is removed from the cells of the sporophyll and stored in the mother-cells, where it remains through their divisions and disappears in the pollen grain as the exine is being formed. The exposed wall of the microsporophyll is but two layers thick.

The reducing divisions in the pollen mother-cells resemble those in the Larix and the reduced number of chromosomes is probably twelve. There is a fairly well developed resting stage after the first division in the mother-cells.

About ten days after the reducing division a division of the pollen grain occurs which separates at once the generative cell from the tube cell. No sterile prothallial cells are formed.

From two to three weeks after pollination, when the pollen tube has grown some distance, the generative cell divides into the central cell and stalk cell, and these move down toward the tube nucleus. The pollen tube reaches the prothallium earlier than in any case previously described, sometimes even before the formation of a cellular tissue in the latter.

The arrangement of the nuclei in the pollen tube is the same as in other conifers.

The central cell has a distinct *Hautschicht* of its own and resembles in outline that of Taxus and the Cupresseae. It divides simultaneously with the division in the central cell of the archegonium, and the two sperm cells thus formed move apart slightly. They are furnished with a dense layer of starch around the nucleus, a peripheral finely granular layer often containing globules of plastic material, and a *Hautschicht.* Its nucleus is densely filled with granular material and has a coarse chromatin recticulum and a nucleolus.

The ovulate cones also begin their development in early fall and continue growing slowly, as the weather permits, through

the winter. At the time of pollination the single megaspore mother-cell may be distinguished. It is filled with starch, as are also the tapetal cells around it. Two reducing divisions occur, but only three cells are formed, the upper of the two first produced not dividing again. The lower of the two potential megaspores resulting from the second division in the lower cell develops into the female gametophyte, the two upper cells disorganizing.

As the spore develops in sprouting, the tapetal cells around it grow and divide, and disorganizing the nucellar cells around them pass the nourishment to the prothallium within. How long this tapetum persists is not certain, but it probably lasts until the prothallium is mature.

The archegonia are arranged as in the Cupresseae. The number of the neck cells vary from two to sixteen or more. The central cell is very long and contains two conspicuous kinoplasmic areas, one at the upper end near the nucleus and the other in the lower end beneath the large central vacuole. When the ventral canal nucleus is cut off the upper of these masses takes part in the division, while the fragmented lower one fills the base of the archegonium with peculiar figures.

A ventral canal nucleus is cut off just before fertilization, but it is not separated from the cytoplasm of the egg and after fertilization moves back toward the center and divides amitotically. It probably assists in nourishing the embryo.

The spindle of the ventral canal cell division is almost entirely of nuclear origin, and the chromosomes are derived largely from the nucleolus. The egg nucleus contains a large amount of granular material, but a chromatin reticulum is always present. This granular material is largely used in the formation of the spindle of the fusion nucleus.

Fertilization occurs about the middle of June, and two or more sperm cells may enter an archegonium. Only one, however, becomes fused with the egg cytoplasm. As a rule one pollen tube fertilizes two archegonia.

The sperm cell with its starch, protoplasm, and nucleus passes through the cytoplasm of the tip of the egg and reach-

ing the female nucleus enfolds it. Its starch is uniformly distributed around the fusion nucleus and passes with it to the base of the archegonia to be included in the cytoplasm segregated off as the proembryo. The larger part of the cytoplasm of the egg takes no direct part in the formation of the embryo, but is digested and used by the latter in its growth.

The first division occurs after the fusion nucleus has reached the base of the archegonium.

Eight free nuclei are formed, which arrange themselves in two tiers, the upper of which generally contains six, the lower two. Cell walls are now formed, but the upper side of the upper tier is left open. This open tier now divides by walls at right angles to the long axis of the archegonium into the rosette of free nuclei above and the suspensors below. The two cells of the lower tier divide at the same time by walls parallel to the long axis of the archegonium, and thus four cells instead of two are produced in one plane.

The suspensors as they elongate may or may not separate, and thus one or several embryos may be formed from one archegonium.

It is thought that the family Taxodieae as now composed is an artificial one; that Taxodium itself must be removed to the Cupresseae, leaving Sequoia and perhaps other as yet imperfectly known genera of the present family Taxodieae to be included in a family of their own under another name.

NOTE.

Since the completion of this work in May, 1901, several papers have appeared bearing more or less closely on certain points here taken up. Miss Ferguson's two papers on fertilization, etc., in Pinus (Annals of Botany, 1901), and Arnoldi's *Beiträge zur Morph. de Gymn.* V. (Bull. Soc. Imp. Nat. Moscow, No. 4, 1900), dealing with fertilization, embryo formation, etc., in the "Sequoiaceen," are important, and would have been referred to frequently in the text had they appeared before this work was handed in. Miss Ferguson confirms Blackman's ('98) statement that the sperm cells of Pinus are furnished with a cyto-

plasm of their own, though the outline of the cell seems to be neither so regular in shape nor so definite in outline as in other groups of gymnosperms. She finds no starch in the male cells, although much is present in the pollen tube. Miss Ferguson also suggests that the "spongy" tissue is useful in nourishing the prothallium, and brings out other interesting points, which must, however, be passed by here.

Arnoldi, in the communication mentioned, finds starch in the sperm cells of Sequoia, Cryptomeria, and Taxodium, and notes its transference to the embryo. He also considers necessary the removal of Taxodium (and several other genera) into the Cupresseae; but on certain points of fact in developmental processes our work does not agree.

In the recent Belfast meeting of the British Association Mr. Harold Wager presented a paper on the function of the nucleolus, in which he is reported as finding that "this body, in the cases examined by him, appears to be intimately connected with the nuclear network, and contains chromatin material which contributes directly to the formation of the chromosomes" (Nature **67**: 20. 1902). These results are of interest in connection with my conclusions.

THE UNIVERSITY OF NORTH CAROLINA,
Chapel Hill, N. C.

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EXPLANATION OF PLATES I–XI.

The abreviations used are: *st*, stalk cell or stalk cell nucleus; *gr*, generative cell; *ptn*, pollen tube nucleus; *cc*, central cell of pollen tube; *sct*, sclerenchymatous cells; *vcn*, ventral canal nucleus; *en*, egg nucleus.

PLATE I.

FIG. 1. Median longitudinal section through young microsporangium; primary archesporium evident as rows of cells. × 400.

FIG. 2. Microsporangium in October; tapetum differentiated. × 300.

FIG. 3. Spindle of first division of microspore mother-cell. × 800.

FIG. 4. Anaphase of same, showing four limbs to chromosomes. × 800

FIGS. 5–6. Chromosomes seen from poles as they approach them; eleven in *fig. 11*. × 800.

FIG. 7. Spindle of second division; mother-cell walls not yet dissolved. × 800.

FIG. 8. Microspore before first division; cell wall not shown. × 800.

FIGS. 9–11. Successive stages in first division of microspore. × 800.

FIG. 12. Pollen grain on tip of nucellus, exine split off. × 800.

FIG. 13. Pollen grain beginning to germinate, exine split off and generative cell swelling out. × 800.

FIG. 14. The same; young stage of germination. × 500.

FIG. 15. Tube nucleus moving down. × 500.

FIG. 16. Different relative position of generative cell and pollen tube than in above. × 500.

FIG. 17. Indication of branching in pollen tube. × 500.

FIG. 18. Generative cell just before commencing to divide. × 800.

FIG. 19. Generative cell immediately after division. × 800.

FIG. 20. Pollen tube with three nuclei. × 280.

FIG. 21. Central and stalk cell of same tube more magnified; stalk cell protoplasm beginning to merge into that of pollen tube. × 800.

FIG. 22. All three nuclei moving down, and close together. × 400.

FIG. 23. Pollen tube slightly older; stalk nucleus has passed central cell and lies by tube nucleus from which it differs in its smaller size; May 15, 1900. × 400.

PLATE II.

FIG. 24. More developed pollen tube; stalk and tube nuclei alike. × 235.

FIG. 25. Tip of ovule; pollen tube has reached megaspore in which no cell walls have yet formed. × 26.

FIG. 26. Tip of pollen tube that has reached the prothallium; nuclei in usual position. × 400.

FIG. 27. Central cell of pollen tube shortly before division; one free nucleus shown. × 800.

FIG. 28. Central cell preparing to divide; free nuclei and protoplasm of pollen tube disorganizing. × 400.

FIG. 29. Spindle of division of central cell. × 800.

FIG. 30. Telophase of the same. × 400.

FIG. 31. Sperm cells separated from each other. × 400.

PLATE III.

FIG. 32. Megasporophyll with the axillary megasporangium rudiment just beginning to show; Oct. 3, 1899. × 95.

FIG. 33. The same; integument beginning to appear; Jan. 4, 1900. × 95.

FIG. 34. Little older megasporophyll and ovule; March 11, 1900, Baltimore. × 95.

FIG. 35. The same; ready for pollination; megaspore mother-cell not yet divided; March 31, 1900. × 80.

FIG. 36. Longitudinal section of sporangia and sporophyll ten days after pollination; placenta now beginning to appear above the ovule; April 3, 1901. × 36.

FIG. 37. Sporophyll and ovule about three weeks after pollination; micropyle closed and placenta becoming prominent; April 22, 1900. × 36.

FIG. 38. Megaspore mother-cell before division. × 800.

FIG. 39. The same; in synapsis. × 1030.

FIG. 40. The same; spindle of first division. × 950.

FIG. 41. The same; older spindle of first division. × 950.

FIG. 42. The same; wall being formed. × 950.

FIG. 43. The same; spindle of second division. × 950.

FIG. 44. Megaspore with the two disorganizing spores above. × 950.

PLATE IV.

FIG. 45. Megaspore before first division; distinct zone of large cells around it. × 400.

FIG. 46. Two cells of the large-celled tissue in division. × 800.

FIG. 47. Germinating megaspore in middle of large-celled tissue; April 29, 1900. × 70.

FIG. 48. Enlarged section of the above-mentioned tissue. × 400.

FIG. 49. Longitudinal section of entire ovule five weeks after pollination. × 36.

FIG. 50. Ovule showing two prothallia. × 70.

FIG. 51. Longitudinal section showing nucleated cytoplasm of wall layer of megaspore, large-celled tissue, and nucellus cells. × 400.

FIG. 52. Large-celled layer around somewhat older prothallium. × 280.

FIG. 53. The same; after-formation of prothallial cells. × 280.

FIG. 54. Young stage, ingrowing cells of prothallium. × 280.

PLATE V.

FIG. 55. Prothallial tubes, after closure of inner ends. × 280.

FIG. 56. Two-celled stage of the same. × 280.

FIG. 57. Tip of young prothallium showing group of archegonium initial cells. × 70.

FIG. 58. Prothallial cell becoming multinucleate. × 400.

FIG. 59. The same with five nuclei. × 400.

FIG. 60. Formation of neck cells in archegonia. × 280.

FIG. 61. Older archegonium; neck cell divided. × 280.

FIG. 62. Still older archegonium; kinoplasmic areas beginning to appear. × 280.

FIG. 63. Nucleus of central cell of archegonium at same age as above. × 400.

FIG. 64. Group of archegonia showing ten in one section, two pollen tubes above; somewhat diagrammatic. × 70.

FIG. 65. Cross-section of group of archegonia, showing seventeen. × 85.

PLATE VI.

FIG. 66. Archegonium showing kinoplasmic masses in central cell; after entrance of plastic stuff from neck cells. × 280.

FIGS. 67–69. Jacket cells in normal activity. × 800.

FIG. 70. Spindle in jacket cell at time of fertilization. × 800.

FIGS. 71–74. Stages of disintegration of nuclei of jacket cells. × 800.

FIG. 75. Lower part of archegonium at time of fertilization showing proteid vacuoles. × 800.

FIGS. 76–82. Successive stages in division of central cell of archegonium; *fig. 76.* × 400; *figs. 77–82.* × 800.

PLATE VII.

FIGS. 83–90. Successive stages in division of central cell of archegonium; *figs. 83–89.* × 800; *fig. 90.* × 280.

FIG. 91. Group of archegonia after formation of ventral canal nuclei. × 36.

FIG. 92. Two archegonia of same group slightly more magnified. × 70.

FIGS. 93–96. Neck cells seen from above. × 400.

FIGS. 97–98. Surface view of megaspore wall showing pits; after formation of young prothallium. × 800.

FIG. 99. Pits of same wall in section. × 800.

PLATE VIII.

FIG. 100. Sperm cell just entering cytoplasm of archegonium tip; its nucleus barely visible in dense starch sheath around it. × 800.

FIG. 101. Sperm cell protoplasm just touching egg nucleus; sperm nucleus not yet in contact with egg nucleus. × 800.

FIG. 102. Sexual nuclei in contact; sperm cell protoplasm beginning to inclose the egg nucleus. × 400.

FIG. 103. Later stage of fusion. × 400.

FIG. 104. Supernumerary sperm cell in tip of archegonium. × 400.

FIG. 105. Later stage of fusion; starch sheath completely inclosing the not yet entirely fused nuclei. × 400.

FIG. 106. Archegonium at the stage above; fusing nuclei moving down and large vacuole broken up. × 70.

FIG. 107. Egg and ventral canal nucleus in tip of an archegonium that did not get fertilized. × 280.

FIG. 108. Ventral canal nucleus at tip of an archegonium of same group as above which has been fertilized and has a two-celled embryo in the base. × 280.

FIG. 109. Ventral canal nucleus in another fertilized archegonium of same group; it has grown out into the protoplasm and is beginning to divide amitotically. × 280.

FIGS. 110–112. Successive stages in growth of ventral canal nucleus in fertilized archegonia; all with young embryos in base; extra sperm cell lying in tip of archegonium in 110. × 400.

PLATE IX.

FIGS. 113–114. Ventral canal cells in archegonia that missed fertilization; others in group have been fertilized. × 280.

FIG. 115. Amitotic division of ventral canal nucleus. × 400.

FIG. 116. Archegonium with 8-celled embryo at base; wells are formed between the cells, but upper cells open above; here there are seven cells in upper tier and only one below; canal nucleus at tip; disorganized layer above embryo. × 400.

FIG. 117. Archegonium with two-celled embryo near base; ventral canal nucleus in division above, and second sperm cell free in tip. × 280.

FIG. 118. Ventral canal nucleus divided, lying at base of archegonium in contact with two-celled embryo, only one of the nuclei of which is shown; embryo surrounded by its starch sheath. × 400.

FIG. 119. Late stage of fusion of sperm and egg nuclei. × 800.

FIG. 120. Spindle of first division of fusion nucleus; large plastin nucleolus unchanged. × 800.

FIG. 121. Two daughter nuclei of fusion nucleus, immediately after division. × 800.

FIG. 122. Preparation for second division. × 400.

FIG. 123. Spindle of second division. × 800.

PLATE X.

FIG. 124. Four-celled embryo; nuclei in usual position, two above and two below arranged tetrad fashion. × 280.

FIG. 125. Embryo of same stage as above, nuclei all arranged at base in one plane. × 280.

FIG. 126. Spindles of third division; plastin nuclei in protoplasm. × 280.

FIG. 127. Eight-celled embryo, six cells above and two below; the upper are dividing, the lower preparing to divide. × 280.

FIG. 128. Abnormal embryo, cut a little obliquely; two of the nuclei have not joined in the tier formation, but have remained a little distance above, and in dividing only one is shown; the other lies beside it in another section. × 280.

FIG. 129. Sixteen-celled embryo in three tiers; six cells in rosette, six suspensor cells, and four below. × 280.

FIGS. 130–132. Abnormal embryos. × 280.

FIG. 133. Sixteen-celled embryo after suspensors have lengthened considerably. × 280.

FIG. 134. Cross-section through a group of seven suspensors. × 400.

FIG. 135. A single suspensor carrying two embryos which are developing separately. × 29.

FIG. 136. Tip of above suspensor with its two embryos more magnified. × 400.

FIG. 137. A two-celled and a three-celled embryo, each on its own suspensor. × 280.

PLATE XI.

FIG. 138. Two four-celled embryos; there are four embryos in the group and four suspensors; three of the embryos are four-celled, and one about six-celled. × 280.

FIG. 139. A three-celled and an about eight-celled embryo; four embryos in group and three suspensors. × 280.

FIGS. 140–142. Three consecutive sections through a group of four

embryos hung by three suspensors; the fourth embryo does not appear in these sections. × 280.

FIG. 143. Two embryos, each on its own suspensor; they are from the same archegonium; there are two others in the group. × 280.

FIG. 144. One embryo on one suspensor, off to itself. × 280.

FIG. 145. Older embryo on much enlarged tip of suspensor; embryonal tubes beginning to appear. × 280.

FIG. 146. Apparently three embryos of a group uniting to form a single embryo; embryonal tubes conspicuous. × 280.

FIG. 147. Older embryo; embryonal tubes extend up about four times as far as shown. × 280.